Volume 29, Number 4, February, 1957

The Yale Journal of Biology & Medicine

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THE YALE JOURNAL
OF BIOLOGY AND MEDICINE

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Subscriptions. THE YALE JOURNAL OF BIOLOGY AND MEDICINE is published in September, November, December, February, April, and June from 333 Cedar Street, New Haven 11, Connecticut, U.S.A. The subscription price for one volume of six issues is $7.50 in the United States and other countries. For students registered in any school of Yale University, the subscription price is $4.00 per volume. Single copies are $1.50 each except for special issues.

Entered as second-class matter January 11, 1929, at the post office at New Haven, Connecticut, under the Act of March 3, 1879.

PRINTED BY THE VAN DYCK PRINTING CO., NEW HAVEN, CONN.

Scope. THE YALE JOURNAL OF BIOLOGY AND MEDICINE publishes original contributions in all fields of medicine, the fields of biology that are related to medicine, and in the history and teaching of these subjects.

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and milled to a yellow flour called turmeric, which is used to color the curries of India. Back in the old days a lot of turmeric went into the staining of leather, but chemistry killed that off long ago.

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JOHN R. PAUL*

Section of Preventive Medicine, Yale University School of Medicine

THE SOVIET PROGRAM FOR MEDICAL RESEARCH, 1956-1960. A BRIEF AND CRITICAL REVIEW OF "THE RESEARCH PLAN OF THE ACADEMY OF MEDICAL SCIENCES U.S.S.R., 1956-60"**†

At the 20th Congress of the Communist party of the Soviet Union, held in Moscow in February, 1956, the sixth five-year plan was presented and accepted. Such plans extend into all walks of life in the U.S.S.R. but particularly in fields of applied science, not the least of which has been the program for work in the medical sciences. This pattern is characteristic of the Soviet Union, and one can more or less count upon the fact that professional, business, and academic activities are supposed to be done, *according to plan,* the basic plans being redrawn every five years.

In the field of medical research such programs are implemented in Russia today under a highly organized system within a large number of research institutes, the staffs of which vary in size approximately from one hundred to one thousand individuals, of whom about one-third are of professional rank. There are three hundred of these institutes in the Soviet Union with great centralization in Moscow, Leningrad, and Kiev. They operate under three administrative systems: (1) the Academy of Medical Sciences, U.S.S.R.; (2) the Ministry of Health, U.S.S.R.; and (3) the Constituent Republics of the Soviet Union. The Academy institutes accentuate *basic* research, the Ministry emphasizes *practical or applied* research, including the production of vaccines and other biological materials, while the institutes under the republics are primarily concerned with *local* problems.

The current (1956-60) plan of the Academy of Medical Sciences, U.S.S.R.,‡ which is the subject of the present review, is concerned primarily with what Soviet scientists and physicians would regard as *basic* and *practical* research work to be accomplished during the next five years. From it one can deduce those subjects which they consider to be the most impor-

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* Professor of Preventive Medicine.

** Problemnyy plan nauchnykh issledovaniy Akademii Meditsinskikh Nauk SSSR na 1956-60 gody, Moscow. Pages 3-111.

† The author was a participant in a Medical Mission to the Soviet Union carried out under the auspices of the U.S. Department of Health, Education, and Welfare, during February and March of 1956.

‡ In the drawing up of this plan the Academy of Medical Sciences has had the collaboration of the Medical Research Council of the Ministry of Health and probably other agencies.

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tant. In this impressive document, of which the English translation is some 125 pages in length, 22 subjects have been listed, some of them very general, some specific. But they are all to be attacked with vigor during the next five years, and it is emphasized at the start that it is the duty of every scholar of the Academy and every citizen to fulfill this five-year plan in complete accordance with the resolutions of the 20th congress of the Communist party in order to reach the great goals of Soviet medical science as well as to meet the requirements of health protection throughout the U.S.S.R.

LIST OF PROBLEMS FOR SCIENTIFIC RESEARCH BY THE ACADEMY OF MEDICAL SCIENCES, USSR, FOR 1956-1960

1. Physiology and pathology of higher nervous activity.
2. Basic mechanisms of the activity of the nervous system and their rôle in regulating the functions of the organism.
3. Mechanism for regulating metabolism, the functions of proteins, and their structure.
4. Mechanisms of action of pharmaceutical substances, and the search for new pharmacological and chemotherapeutic materials.
5. Morphology of tissues.
6. Principles of the prophylaxis and treatment of hypertensive disease, arteriosclerosis, and coronary insufficiency.
7. Surgery of the lungs, heart, and large blood vessels.
8. Surgery of the nervous system.
9. Pathogenesis, prophylaxis, and treatment of basic diseases of the nervous system.
10. Malignant tumors.
11. Etiology, prophylaxis, and treatment of tuberculosis.
12. Epidemiology, prophylaxis, and treatment of intestinal infections, particularly dysentery.
13. The protection of the health of women, mothers, and newborn children.
14. Etiology, epidemiology, prophylaxis and treatment of acute infections of childhood (scarlet fever, measles, whooping cough, and diphtheria).
15. The physiological basis of rational nutrition for healthy and ill persons.
16. The hygiene of working conditions and the prophylaxis of occupational diseases.
17. The hygiene of urban or crowded communities.
18. Laws of the variability of microbes and their importance in biology and medicine.
19. Etiology, epidemiology, and immunology of viral infections, particularly influenza.
20. New antibiotics and synthetic chemotherapeutic agents.
21. Epidemic poliomyelitis.
22. Pediatrics.

Most of this document is given over to an explanation or an elaboration of these headings, and these elaborations will be given and commented upon in a very abridged fashion in this report. It is not clear whether these subjects have been listed in order of their priority but it is unlikely that the

first two subjects, dealing as they do with the physiology and pathology of the central nervous system, occupy this prominent position by pure chance.

Item 1, *Physiology and pathology of higher nervous activity.* In essence this represents the continued growth of the work and concepts of the late Professor I. P. Pavlov, and the adaptation of his doctrine to problems of physiology and clinical medicine. This Pavlovian philosophy penetrates a number of branches of Soviet medicine today, going far beyond that of neurophysiology per se, into the fields of immunology, endocrinology, clinical medicine, and psychiatry. Within the Soviet Union the influence of Pavlov's work on conditioned reflexes as a basis for human behavior in both normal and psychasthenic patients overshadows that of Freud.

According to the plan the lines along which this research is to proceed are several, and, as with all of these 21 other major subjects, the project is to be directed by one Institute, in this instance the *Institute of Experimental Medicine* in Leningrad, but certain of its aspects are to be farmed out to as many as a dozen or more laboratories or clinics, located in different places.

Particular aspects in which this research is to be pursued are concerned with: the nervous activity of the cerebral cortex, the dynamic localization of functions within the cerebral cortex, and with the attempt to elucidate the mechanism of basic nervous synthesis including the processes of formation of the conditioned reflex. The program will also deal with the pathology of higher nervous activity and is actually a study of those pathological states which can be expected to produce disturbances of higher nervous activity. This calls for the creation in animals, notably monkeys, of various pathological states in the nervous system, and for the elaboration of an experimental policy as to the prophylaxis of these states.

One of the laboratories to which this problem has been assigned is the medical biological station at Sukhumi on the Black Sea (in the Republic of Abkazia). Here a large colony of primates, consisting of monkeys and chimpanzees, is kept and bred under more or less natural conditions and is available for various kinds of experimental work. The production of nervous states, i.e., neuroses, etc., in the monkey has long been part of a program of research at this institute where by the utilization of normal conditioned reflexes the investigators have been able to establish neuroses in these animals, some of which appear to be irreversible.

This work on the pathology of nervous activity also envisions studies on growth and maturation to be correlated with the development of nervous activity in infants, pre-school, and school-age children. It is also to extend to studies on the development of reflex activity in the embryonic and postnatal periods of life, on the formation of individual traits as regards nervous activity, and on the effects of environment on the pattern of higher nervous

activity in children during the first three years of life. Much of this is being done with the idea of determining the degree to which a system of education may alter the situation.

Item 2, listed as *Basic mechanisms of activity of the nervous system,* is the second phase of this project in neurophysiology and neuropathology. The work here deals with the principle of dominance as a working mechanism of the activity of the nervous centers in the normal nervous system. This will concern the processes of inhibition, the physiology and morphology of the vegetative nervous system with particular emphasis on cortico-visceral interrelationships in normal and pathological states: the regulation of circulation, respiration, and digestion.

Item 3 on the list is labelled *Functions of proteins,* their structure and their metabolism. This obviously can take many forms but here again the rôle of the nervous system has received considerable emphasis as a mechanism for the regulation of the activities of various enzymes within the organs or tissues of the body, which may in turn influence the amino-acid composition of individual proteins.

Such emphasis upon the nervous control of bodily functions and body composition indicates with what respect this central nervous control is viewed not only by Soviet physiologists but by biochemists.

4. Research in *Pharmacology* is to continue along the lines which they claim to have been following for the past five years, namely, in the development of theoretical matters and the discovery or chemical synthesis of new pharmaceuticals. Attention is to be paid here to the extraction of active ingredients from plants which grow in the Soviet Union.

5. Under the heading of *Morphology* it is pointed out that the examination of tissue structure had been the center of scientific attention during recent years but the earlier emphasis on structural changes in organs and cells has given way somewhat to the study of their pathological physiology. Nevertheless, the study of structural changes in tissues and cells is not to be neglected and here again neuro-anatomy and neuropathology seem to have a high priority. There is also to be an interest in the pathology of fibrous structures, the pathology of radiation injury, of the infection process, of the cardiovascular system, and of neoplasms. One whole major section is given over to the pathology of occupational diseases; another to that of vascular disease.

6. *Hypertension and arteriosclerosis.* As to work which they consider desirable on the etiology and pathogenesis of hypertension, their primary approach carries with it the intent to clarify the nature of nervous and humoral factors and their rôle in etiology. This will include clinical investi-

gation on the treatment and prophylaxis of hypertension in patients, using new drugs in particular.

Under the heading of arteriosclerosis and coronary artery insufficiency (presumably atherosclerosis in our terminology), efforts are to be directed not only towards the analysis and the rôle of the nervous and humoral factors but of their metabolic features, which are not to be limited solely to disorders in lipoid-fat metabolism. These are aimed essentially toward devising principles for the prophylaxis and treatment of hypertension, arteriosclerosis, and coronary artery insufficiency. There is no apparent mention of the epidemiological approach in this plan, for here, as in other non-infectious diseases, clinical epidemiology does not seem to have been taken up, or at least it is not mentioned as such among the other plans of investigation.

7. Research in *Surgery* calls for work on the lungs, heart, and large blood vessels. There are prominent and ambitious plans here for the development of thoracic surgery, particularly as it is pointed out and stated in the plan that thoracic and cardiac surgery does not hold the place it might, the reason being that facilities do not yet exist in the Soviet Union which will supply the needs of modern surgery for special instruments and equipment, anesthetics, and drugs. The new program calls for improvement in this situation. One of the first requirements is concerned with research on anesthesia; a long list of plans dealing with general and special problems in lung surgery and of surgery of the heart and the great vessels comes next.

8. *Neurosurgery* is another major item. Here is listed: research on problems of trauma, tumors and inflammation of the central nervous system to be coupled with studies on the reaction of the brain to local injury.

9. In the field of *General neurology* work is to be carried out on the pathogenesis, prophylaxis, and treatment of diseases of the central nervous system which includes a variety of brain lesions as well as viral infections, peripheral neuritides, and neuroses.

10. A major heading is devoted to *Malignant neoplasms,* and, considering the number of large institutes wholly devoted to this field, it is surprising that this does not appear earlier on the list. The Soviet pattern of cancer research will follow that to be found in this country. It is an attack over a wide front dealing with viral, chemical, and hormonal theories on the etiology and pathogenesis and therapy of tumors. This ramifies into many fields and is to be carried on in many institutions. The plan includes the prophylaxis of tumors, methods of diagnosis and therapy (chemical, physical, immunological, and hormonal), and even the administrational aspects of organizing the fight against cancer. It reflects a tremendous interest in

the whole subject and the desire of Soviet scientists to be in the forefront of a world-wide attack on cancer.

11. *Tuberculosis* occupies a whole section, reflecting the fact that this disease is still an important cause of illness in the Soviet Union. Research is to be concerned with the prophylaxis, pathogenesis, clinical aspects, and therapy, as well as the epidemiology of tuberculosis and the organization of anti-tuberculosis work.

12. Next comes the *Epidemiology, prophylaxis, and treatment of intestinal infections,* especially dysentery. These enteric diseases apparently continue to be very important causes of illness throughout the Soviet Union. Particular attention is to be paid to the prophylaxis of these disorders, and the creation of new preparations for immunization among which are the so-called polyvalent, high-activity preparations of bacteriophage and a study of its effectiveness. One feature is concerned with "the laws of the dysentery epidemiological process," i.e., the study of factors causing the seasonal rise in dysentery infections and the uneven distribution of infection among age groups; its spread under rural conditions, and the rôle which hygienic measures play in its control.

13. There is a section on *Maternal and child welfare* calling for research on: the handling of cardiovascular disorders during pregnancy, childbirth and the puerperal period, as well as the toxemias of pregnancy, the regulation of labor, and the protection of the newborn, and studies on fertility. One specific project is a critique on the broad scale use of x-ray diagnoses or x-ray therapy in females, notably pregnant females, as to the effect of radiation energy on the ovum, the embryo, and the female organism. It was specifically stated that more effective measures must be developed to avoid radiation disease and to treat it.

14. *Infectious diseases of childhood* come next. Specifically mentioned are scarlet fever, measles, pertussis, and diphtheria. It may be of some interest to note that scarlet fever is listed first, a disease which in many European countries today is of so much less importance than it used to be.

This program is to cover work on the etiology, epidemiology, prophylaxis, and therapy of childhood diseases, which seem to be listed in a certain order of priority. Pertussis receives special attention both from the standpoint of improving methods of diagnosis and the development of vaccines, of which they have various types. The work on scarlet fever calls for the development of an effective method of active immunization with a study of the immunogenic properties of various streptococcal antigens. Noticeable in this first mention of a streptococcal disease is the absence of work dealing with the possible association of rheumatic fever with streptococcal illness.

In the plans for the continued control of diphtheria mention has been made of the fact that active immunization and improvements in therapy have resulted in a sharp drop in illness and death rates due to that infection.*

Considerations with regard to measles indicate that sero-prophylaxis of this acute infection is widely practised in the Soviet Union, but the plan points out that this method of passive immunization is of limited significance from the standpoint of reducing the total rate of infections. The serum used is mainly convalescent serum, and it is pointed out that this carries another shortcoming because of the possibility of transmission of viral infections such as hepatitis. It is further mentioned that the danger of this complication can be eliminated by the use in measles of gamma globulin, i.e., human serum concentrate. The extent to which gamma globulin is available in the Soviet Union is not stated, but the chances are that the supply is limited. In keeping with the program of control of other infectious diseases work on vaccines to produce active rather than passive immunity is in progress.

15. Another basic program is concerned with *Nutrition* and its physiological basis for healthy and ill persons. This covers work on the prophylactic nutrition of industrial workers and also includes a section on the effects of residual quantities of poisonous chemicals used in agriculture and their bearing on crops exposed to such chemicals when the agricultural product serves as human food.

16. Another whole section illustrates the Soviet interest in *Industrial disease* and in improvements in the control of occupational hazards. Among these occupational diseases are those which affect workers engaged in agricultural pursuits, and those resulting from exposure to dust, industrial intoxications, and radioactive substances which may be used in industry. Particular attention is to be placed on the dynamics of radioactivity in populated places and in industrial installations. Part of this same project is concerned with the effect on the human organism of certain physical factors in his environment. It is pointed out that the increasing use of x-ray techniques, electronics, high-frequency currents, and pneumatic instruments all need further study as to what the possible biological effects on the human organism may be.

* Vital statistics on the actual incidence or prevalence of diseases in the Soviet Union are not mentioned in this report or indeed in hardly any of the Soviet medical publications. It is not known whether this information is regarded as secret, or whether the fact that there has not been a general population census in the Soviet Union since 1939 has rendered the compiling of accurate rates on the prevalence of illness so difficult that statistics are simply not available.

The physiology of the laborer is to receive consideration, i.e., processes of adaptation required for men who work in conditions under which they are exposed to high-level noise, vibrations, ultra-high-frequency radiations, and radioactive isotopes. These are studies designed to improve the capacity of the human body to work and to maintain health at a high level under conditions which tend to give rise to stress. Included in the program is sanitation of the air with attention being paid to the effects of industrial wastes, or harmful substances in the atmosphere, of ionizing radiations in the atmosphere of large cities and industrial centers and the penetration of the organism by aerosols. This is directed towards the production of methods for the deactivation of gaseous wastes and the protection of the water supply. Housing and lighting are also included in this program.

17. Another section could be termed *Population research.* It deals with the acclimatization of populations within the newly colonized regions of the USSR reflecting the fact that mass migrations have been carried out.

18. The subject of *Microbial variation* is given a whole heading, indicating its importance in biology and medicine. At first glance this can be considered basic research indeed. It deals with the laboratory production of new species or subspecies, or subtypes of microörganisms, bacteria, and viruses, and the possible relation of such changes to what they call the epidemic process. This form of research is not only concerned with methods of producing variation or mutants but deals with the action of drugs on microbes and drug resistance. It is also being oriented towards the production of nonvirulent variants which might be useful in vaccines. This is particularly important because Soviet medicine uses live, attenuated bacterial and virus vaccines on man more extensively than is the situation in this country.

19. *Viral infections* occupy another whole division. The plans, which occupy at least seven pages of the report, deal with etiology, epidemiology, and immunology, and in the opening statement the reader is reminded that Soviet scholars have already opened up the field of a number of new infectious diseases such as hemorrhagic fever (of which there are several varieties) and "two wave" meningo-encephalitis which is apparently a variant form of Russian spring-summer encephalitis. The number of other vital diseases to be investigated are many, but not the least member is viral hepatitis, known by them under the name of Botkin's disease. Others to receive special attention will be measles, rabies, trachoma, and in particular influenza. Research upon influenza represents a development which has been going on for many years in the Soviet Union. It is concerned with experimental influenza in animals, variations in influenza viruses, improve-

ments in clinical diagnosis, vaccine production, and studies on the basic defense mechanisms of influenza, as well as its epidemiology in man.

20. *Antibiotics* and synthetic chemotherapeutic agents for use in therapy occupy a whole section. None of the antibiotics which they have developed is actually designated by name in this review, although the Russians have developed at least one of these which is being used in that country along with others developed in East Germany. In the planning for extensive work in this field many institutes are named as collaborating agencies. Included among the bold objectives is the discovery or the development of antibiotics for the treatment of virus diseases, such as influenza, poliomyelitis, hepatitis, and measles.

21. *Epidemic poliomyelitis* occupies a whole section. This is surprising because that disease has not been particularly common in the Soviet Union or at least recognized as being common. It would seem that the importance of poliomyelitis in other parts of the world has spurred Soviet medical scientists into special action in this field.

First on the list of desiderata for them is the development of a specific vaccine for the prophylaxis of poliomyelitis, i.e., the mass production of a vaccine of the Salk type, and the study of the various strains of polioviruses to be incorporated into their formalinized vaccine. Nothing is mentioned about the use of live, attenuated virus vaccines in this disease. The plan also calls for improvements in the laboratory diagnosis of poliomyelitis and the study of its epidemiology. Clinical investigations which include work on the after-care are also contemplated. Notable for its absence is any mention of research in the development of mechanical aids for those patients with respiratory paralysis.

22. The final section is given to *Pediatrics,* with individual sections on child hygiene, nutrition, childhood infections, and blood dyscrasias. Here for the first time rheumatic fever is mentioned. It is planned to study: the pathogenesis of rheumatic fever, the rôle of chronic tonsillitis and the latent clinical forms. For the therapy of active rheumatic fever clinical studies are planned on the synthetic preparation of drugs (butadione) and steroid hormones (ACTH) and cortisone. For the prevention of rheumatic fever scientific research is advised to improve methods of combatting focal infections, to evaluate the use of anti-rheumatic chambers, and the rôle of forest schools. It would seem that the concept of Group A hemolytic streptococcal infections playing an etiologic rôle in rheumatic fever, if recognized, is at least not mentioned in these plans.

Critique. In summary, then, it would appear that an over-all plan of this kind, so extensive and so ambitious, covering as it does basic and applied

work in the whole field of medical sciences is a phenomenon in itself. If comparisons are to be sought, it would perhaps be better to compare this program with one which might be evolved within European countries as opposed to one from the United States. For within Scandinavian countries, Germany, France, and Italy a national policy for medical research to be carried out in one or more government institutions is more familiar than it is in this country. In other words, although the general pattern of this type of approach may be less familiar to us than it would be elsewhere, this should not detract from our judgment of its merits or demerits.

As to the calibre or stature of the proposed research program the reviewer is certainly incompetent to make a blanket estimate considering the variety of different subjects involved, covering as they do medicine, surgery, pediatrics, hygiene, and preventive medicine as well as most of the so-called basic medical sciences of physiology, pharmacology, microbiology, etc. Some of the proposed research appears to be almost ultramodern; some could be regarded as outmoded. This is not an excuse to look down one's nose, for the reviewer is reminded of the fact that brilliant discoveries (in contrast to developmental researches) are seldom made in fashionable fields.

Some of these research programs which are to be pursued by Soviet scientists during the next five years reflect an advanced and logical stage of development in disciplines in which they have already had long and valuable experience. Particularly is this true of the field of neurophysiology. Pavlov's mantle has fallen on the shoulders of a number of his disciples and the adaptation of his techniques and philosophy to neurophysiology and to psychology is still given a high priority by Soviet medical science. This concept also penetrates into five or six of the 22 main subjects to be investigated.

The experimental work in virology and its accompanying epidemiological survey plan, particularly that concerned with influenza and with the Russian spring-summer viral encephalitides, is timely and by no means inferior to some of that going on in Europe and the United States today.

On the other hand, there are noticeable gaps to which one can easily point, such as a dearth of plans calling for the use of tissue culture as a means of propagating a variety of viruses. Apparently this method, which has so revolutionized virology in the United States, England, and certain European countries, has only just begun to be appreciated in Russia.

As to what might be called other omissions—subjects which might appear if, for instance, a program of this kind had been drawn up in the United States in 1956—the reviewer is on shaky ground, for it is clear that interpretations of first things first would differ in different lands. The rarity of research work on rheumatic fever in the Soviet plan is, however, worth mentioning. Perhaps this is because Soviet physicians are not at all agreed

as to the pathogenesis of this disease. Their mention of the use of "forest schools" as a prophylactic measure against rheumatic fever would seem to reflect an approach which could be regarded here as outmoded. Endocrinology and geriatrics do not seem to receive much attention as major subjects of research. The rather limited use by the Soviets of the epidemiological approach to noninfectious diseases such as heart disease and cancer, which is only just beginning in the United States, might be mentioned here because actually it is the kind of research that could be more readily carried out in Soviet Russia than in many other places. One might also say that the whole variegated field of "intramural clinical investigation," i.e., the intensive study of sick patients, which is today so characteristic in this country of full-time medicine, figures only slightly in the Soviet Plan. This an approach which requires highly organized hospital staffs consisting of residents, internes, and Fellows who work on the wards and in laboratories within the hospital. This type of personnel and these facilities are not generally existent as such in major Soviet hospitals.

Part of this program of research has been obviously prepared to indicate that Soviet Medicine and Soviet Medical Research are not behind the times and are not to be caught napping. The heavy emphasis upon the use of radioactive material and of isotopes in research, of the dangers from exposure to radioactive substances, all suggest an awareness of the value, dangers, and newness of the atomic age.

Another feature, which is thoroughly genuine, is their emphasis on preventive as compared with curative medicine. Prophylactic measures occupy a prominent position throughout many of these plans. This is reflected by their extensive plans for: research on the control of communicable disease, the protection of populations against radioactive materials, and the emphasis, as is natural in the Soviet Union, on industrial hygiene and the protection of the laborer. Child welfare also stands out prominently among the lists as an important aspect of preventive medicine.

Soviet scientists are well aware that scientists in the United States tend to regard certain aspects of Soviet biological as well as medical science as flimsy. This stems from the severe down-grading which Soviet science received some years ago from doctrines promulgated by men such as Lysenko in the field of genetics. In Lysenko's case, a government order backed up a personal and, to say the least, a controversial opinion which was all too reminiscent of Hitler's arbitrary decrees about scientific thoughts. This episode occurred about ten years ago. Today in Russian scientific circles Lysenko is not a happy subject of conversation. Official scientific doctrines established by decree no longer seem to be the fashion,

and widespread differences of opinion are far more openly expressed among medical scientists than was apparently previously admitted.

One of the questions frequently asked of those who have recently observed medical research in the Soviet Union is whether or not there is any chance for individual effort and initiative to express itself within the framework of their totalitarian system and long-term plans such as this one. Soviet medical authorities go to some length to reassure visitors from outside countries that there *is* room for individual initiative and talent, in spite of their profound deference to plans. As an example, it is frequently stated that a young scientist may often be given the opportunity of developing an idea of his own and may even carry out preliminary experiments on a subject of his own choosing in the laboratory where he happens to be placed. If these experiments are promising, he is allowed to report his results before one of the medical societies or its branches, i.e., the Physiology Section, the Pathology Society, etc. If the work receives favorable comment there and it seems to deserve to be pursued further, an outline for future work in the field is presented to the appropriate committee of the Academy of Medical Sciences or the Research Council of the Ministry of Health. And here again, if it is received favorably, it may be eventually written into a plan of research, such as the one which is here received. Furthermore, individual prizes are given to individuals who have shown originality and brilliance in their scientific work and ideas. This, it is pointed out, is a stimulus for such initiative on the part of a young scientist and is supposed to reflect the fact that *individual achievement* in a field in which the state is interested is in full keeping with what might be termed the Soviet religion.

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THE EFFECTS OF CORTISONE AND CHEMICAL INFLAMMATION ON EXPERIMENTAL MUCORMYCOSIS (RHIZOPUS ORYZAE INFECTION)‡§

The fungus Rhizopus oryzae which belong to the order *Mucorales* is a unique test agent to study the effects of metabolic alterations on infection since it behaves differently in the metabolically normal and abnormal host. This fungus was originally isolated at autopsy from a diabetic patient who died with cerebral mucormycosis.[2] We have shown in previously reported experiments that inoculation of the fungus in rabbits with acute alloxan diabetes produces extensive nasal, pulmonary, and cerebral lesions which closely resemble those of mucormycosis in man.[3] Inoculation of the fungus into normal rabbits produces only rare and minute circumscribed lesions at the site of inoculation. Similar experimental results have been obtained by other investigators who employed another fungus of the order *Mucorales*, Rhizopus arrhizus.[1] We have found further that hyperglycemia induced by continuous intravenous infusion and not associated with diabetes mellitus produces in rabbits nasal and sometimes pulmonary lesions which, although more numerous and extensive than the lesions in normal animals, do not approach the extent and severity of the lesions in rabbits with acute alloxan diabetes.[4] The fungus lesions in both diabetic and hyperglycemic nondiabetic rabbits have in common striking degenerative changes in the polymorphonuclear leukocytes consisting of nuclear pyknosis and karyorrhexis.

The following experiments attempt to define further the metabolic alterations which are essential in the pathogenesis of mucormycosis. Because of the known decrease of host resistance to infection following cortisone administration the effect of this hormone on experimental mucormycosis in rabbits was studied in one series of experiments. In other experiments, the

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‡ Aided by Grant A-954 from The Public Health Service, Department of Health, Education and Welfare.
§ Presented at the fifty-third annual meeting of the American Association of Pathologists and Bacteriologists in Cincinnati, Ohio, April 26-28, 1956.

effect of a pre-existing inflammatory process on this fungus infection was investigated since autopsy findings in man suggested the possibility that an inflammatory focus might produce local conditions suitable for the proliferation of the fungus in tissues.

CORTISONE ADMINISTRATION

METHOD

The preparation of the fungus spore suspension,* the inoculation procedure and the determinations of blood and urine sugar and urine acetone were the same as described previously.[8]

Five groups of male rabbits weighing between 1900-2300 gms. were given 5 mgm. of intramuscular cortisone twice daily for periods of 4, 6, 10, 15 and 23 days. Groups I-IV comprised 3 animals each, Group V consisted of 5 rabbits. Forty-eight hours before termination of the experiment a suspension of Rhizopus oryzae spores was instilled into one nostril. Blood sugar determinations were performed twice daily on the rabbits of Groups I-IV and once daily except on week-ends on the animals of Group V. Urine sugar and acetone were determined once daily on the rabbits of Groups III, IV, and V. At the end of the experiments the animals were sacrificed by injection of air into the marginal ear vein. Complete autopsies including fungus cultures from the nose and lung were performed on all animals.

Controls consisted of 4 male rabbits weighing between 2,000-2,300 gms. which were subjected to the same experimental conditions as Group V except for fungus inoculation. Forty-eight hours before termination of the experiment, these animals were given 1.5 cc. of sterile normal saline into one nostril.

RESULTS

The results of this experiment are summarized in Table 1.

Fungus lesions were present in 10 of the 17 rabbits. Nasal lesions were found in 2 of the 3 rabbits in Groups I and III, in all 3 animals of group IV, and in 3 of the 5 rabbits in GroupV. Pulmonary lesions were encountered only in 2 animals of Group IV. No fungus lesions were noted in Group II. Fungus cultures of nose and/or lung were positive in all 17 rabbits.

The nasal lesions consisted of multiple minute superficial circumscribed mucosal ulcerations with some budding spores, hyphae, and mycelia and a moderate infiltration of normal-appearing polymorphonuclear leukocytes (Figs. 1 and 2). There was no involvement of bone or blood vessels. The pulmonary lesions consisted of rare minute circumscribed foci of inflammation in the alveoli and alveolar ducts with a few degenerating spores sur-

* We are indebted to Dr. Libero Ajello, Chief, Mycology Section, Communicable Disease Center, Department of Health, Education and Welfare, Chamblee, Georgia, for the preparation of the spore suspensions.

rounded by normal appearing polymorphonuclear leukocytes (Fig. 3). No vascular involvement was present.

All rabbits including the controls showed marked cortisone effect, consisting of glycogen infiltration of the liver and atrophy of the adrenal cortex. The effect of the hormone was particularly pronounced in the animals of Groups IV and V which in many instances also showed the nodular

TABLE 1. EFFECT OF CORTISONE ON RHIZOPUS ORYZAE INFECTION IN RABBITS

Rabbits		*Days*	*Fungus lesions*		*Fungus cultures*	
Group	*Number*	*cortisone*	*Nose*	*Lung*	*Nose*	*Lung*
I	3 (777-779)	4	2	..	3	3
II	3 (781-783)	6	..	..	3	3
III	3 (784-786)	10	2	..	3	3
IV	3 (787-789)	15	3	2	3	1
V	5 (840-844)	23	3	..	5	..

Total number of rabbits	17
with fungus lesions	10
with positive fungus cultures	17

glomerular lesions described in rabbits receiving cortisone over long periods.[8]

All animals of Groups I and II and one rabbits of Group III showed a slight rise in blood sugar with an increase of 40-50 mgm% over the baseline values. The animals of Groups IV and V as well as 3 of the saline-inoculated rabbits revealed a definite persistent elevation of the blood sugar levels ranging between 140-390 mgm% with an average value of 239 mgm%. No significant hyperglycemia was encountered in one of the 4 controls. Appreciable glycosuria occurred most of the time in all animals tested but acetonuria did not occur.

Gross and histologic post-mortem studies including fungus cultures of the rabbits receiving cortisone and instilled intranasally with saline revealed only the already mentioned changes attributed to cortisone administration.

CHEMICAL INFLAMMATION

METHOD

The details of the preparation of the fungus spore suspension and of the inoculation procedure have been described previously.[8]

Four groups of male rabbits weighing between 1850-2500 gms. were lightly anesthetized with intravenous Nembutal and instilled into one nostril with 0.3 cc. of croton oil. Groups I and IV each consisted of 4 rabbits and Groups II and III each of 5

TABLE 2. CHEMICAL INFLAMMATION (CROTON OIL) AND RHIZOPUS ORYZAE INFECTION IN RABBITS

Rabbits		*Inoculation (Hrs.)*		*Fungus lesions*		*Positive fungus cultures*
Group	*Number*	*After Croton oil*	*Before autopsy*	*Nose*	*Lung*	*Nose and/or lung*
I	4 (893-896)	48	48	1	2	3
II	5 (897-901)	48	96	..	..	5
III	5 (902-906)	96	48	..	2	4
IV	4 (907-910)	96	96	..	1	3

Total number of rabbits	18
with fungus lesions	6
with positive fungus cultures	15

animals (Table 2). Forty-eight hours after the instillation of croton oil a suspension of Rhizopus oryzae spores was given into the already injured nostril of the rabbits comprising Groups I and II. The animals of Group I were sacrificed 48 hours and those of Group II 96 hours after fungus inoculation. The rabbits of Groups III and IV were inoculated with the fungus spore suspension 96 hours after croton oil administration and were sacrificed 48 hours (Group III) and 96 hours (Group IV) later. Eight male rabbits served as controls. These received 0.3 cc. of croton oil into one nostril but were not inoculated with fungus. Two animals were sacrificed 48 hours after croton oil instillation, 3 after 72 hours, and the remainder after 96 hours. Complete autopsies including fungus cultures from the nose and lung were performed on all animals.

RESULTS

The results of this experiment are summarized in Table 2.

In all 18 rabbits croton oil produced extensive lesions at the site of inoculation in the nose and in 11 of these animals pulmonary lesions second-

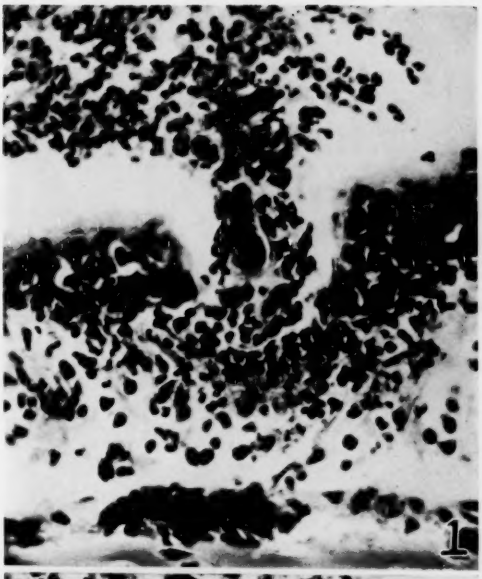

FIG. 1. Typical nasal lesion of rabbit treated with cortisone showing small mucosal ulceration with mycelia and leukocytes. Giemsa, x405.

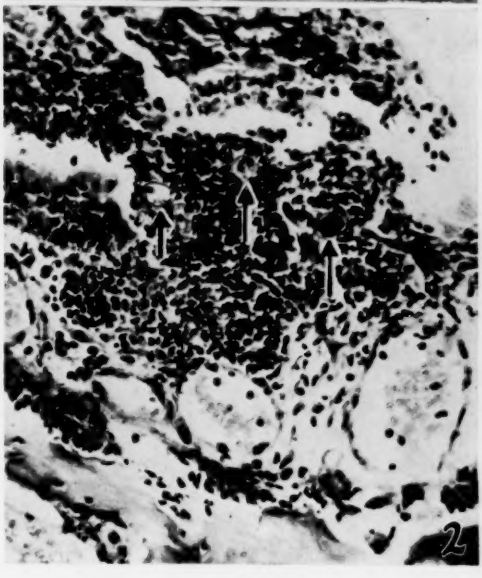

FIG. 2. Largest nasal lesion encountered in rabbits receiving cortisone. Several mycelia (arrows) among leukocytes. Giemsa, x230.

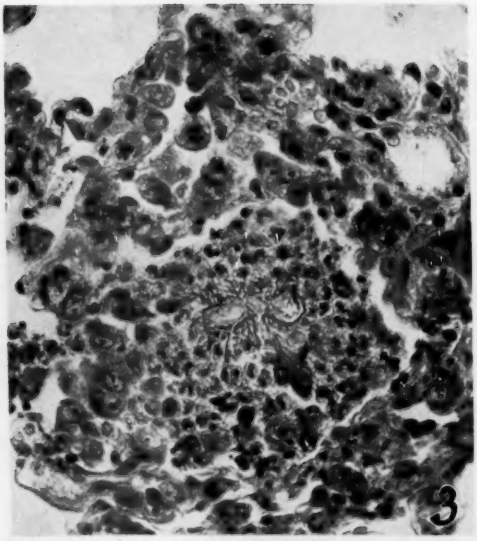

FIG. 3. Cortisone-treated rabbit. Pulmonary lesion with degenerating spores (center) surrounded by leukocytes. Giemsa, x460.

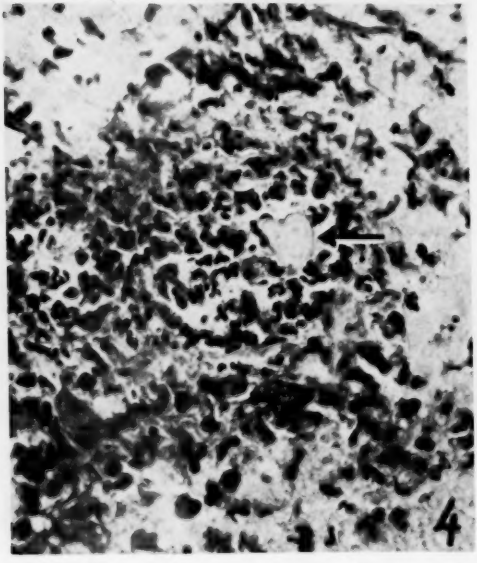

FIG. 4. Nasal lesion produced by croton oil with degenerating spore (arrow) surrounded by leukocytes. Giemsa, x469.

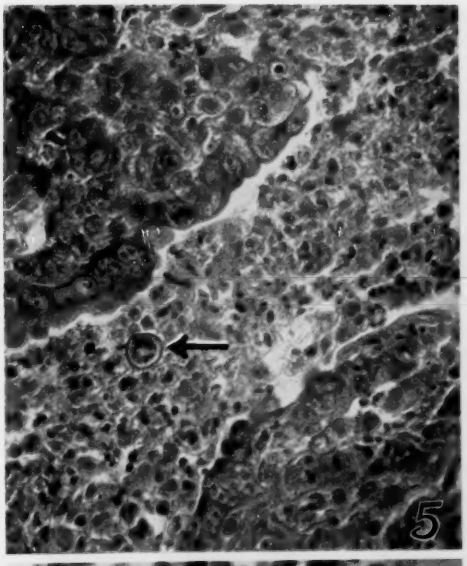

FIG. 5. Viable spore (arrow) in exudate of alveolar duct immediately adjacent to lung abscess produced by croton oil. Giemsa, x465.

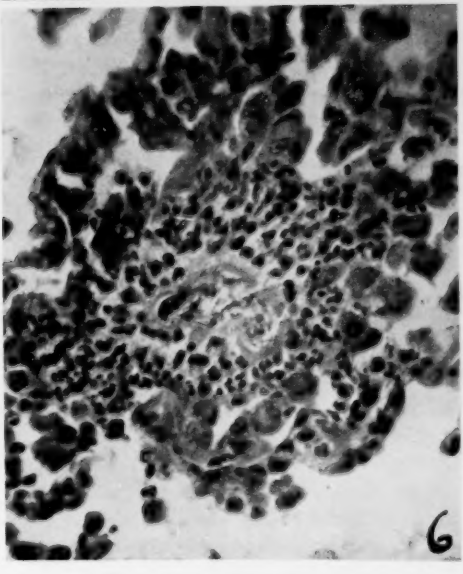

FIG. 6. Lung of rabbit instilled with croton oil. Degenerating hypha (center) surrounded by leukocytes. Giemsa, x460.

ary to aspiration of the chemical had occurred. The nasal lesions consisted of large mucosal ulcerations with necrosis of the deeper tissues which sometimes involved the bone. The lungs showed areas of bronchopneumonia frequently with extensive abscess formation. All lesions revealed a marked inflammatory response. Repair was pronounced in all lesions of 96 hours and longer duration.

No lesions which could be attributed to the fungus were found in any of these animals. In a few instances the fungus could be identified histologically in the lesions produced by croton oil. No fungus was seen in any of the nasal lesions except in one rabbit of Group I. Fungus was observed in the pulmonary lesions of 2 rabbits in Groups I and III and in one animal of Group IV. Fungus cultures from nose and/or lung were positive in 15 of the 18 rabbits.

The histological demonstration of the fungus was difficult, and the agent was found solely in association with the croton oil lesions. Only rare spores were encountered, all of which except one were degenerating (Fig. 4). This viable-appearing spore was present in an alveolar duct (Fig. 5). A single degenerating hypha was encountered in a pulmonary lesion of another rabbit (Fig. 6). The viable appearing spore and the degenerating hypha both occurred in the pulmonary lesions of rabbits of Group I. Wherever the fungus could be seen, it was surrounded by a vigorous inflammatory response consisting chiefly of normal-appearing polymorphonuclear leukocytes.

The croton oil induced lesions in the 8 control rabbits closely resembled those of fungus-inoculated animals in size, distribution, and histological appearance.

DISCUSSION

Our findings indicate that cortisone in the amount given in this study does not produce conditions suitable for the development of mucormycosis with unrestrained proliferation of Rhizopus oryzae in the tissues. The hormone, however, alters the host resistance to the fungus as shown by the increased incidence of the fungus lesions. These are about three times more common in the hormone-treated rabbits than in the normal controls. Furthermore, budding spores, hyphae, and mycelia occur regularly in the lesions of the hormone-treated animals but are not generally found in the fungus lesions of normal controls. Despite this enhanced proliferation of the fungus, the lesions in the cortisone-treated rabbits resemble those of the metabolically normal controls and do not approach the extent and severity of the fungus lesions in rabbits with either infusion hyperglycemia or acute alloxan diabetes. Our observations differ from those reported by Baker and

associates.[1] These investigators found that experimental peritoneal Rhizopus oryzae infection in cortisone-treated mice, rats, and guinea pigs spreads to extraperitoneal organs to a much greater extent than in normal controls. More hyphal forms of the fungus were noted in the lesions of the hormone-treated animals. It was concluded that cortisone has a tendency to promote spreading of the fungus in the animal body.

The unusual scarcity of visible spores and the virtual absence of fungus growth in the extensive inflammatory lesions produced by croton oil indicate that a pre-existing focus of inflammation in a metabolically normal host does not provide an environment suitable for the development of this fungus infection. Histologically demonstrable fungus in the croton oil lesions was considerably less frequent than in the tissues at the inoculation site of metabolically normal animals. It appears, therefore, that a pre-existing inflammatory reaction not only restrains the growth of the fungus inoculum, but also destroys spores.

The scarcity of the fungus in these lesions may be related to the presence of large numbers of polymorphonuclear leukocytes which appear normal. In contrast, the polymorphonuclear leukocytes in the fungus lesions of rabbits with systemic metabolic alterations such as acute alloxan diabetes or infusion hyperglycemia are also present in large numbers but show marked degenerative changes. Dubos considers it likely that metabolic changes in the internal environment of the leukocyte affect its bactericidal function.[5] The increased susceptibility to mucormycosis of cortisone-treated rabbits may in part be related to hormone-induced metabolic changes in the polymorphonuclear leukocytes. Martin and associates have shown that cortisone reduces the lactic acid production by the leukocyte *in vitro*.[6] It has further been demonstrated that *in vitro* the polymorphonuclear leukocytes of diabetic patients have impaired glycolytic power and also form less lactic acid.[7]

Our findings again emphasize the important rôle of the polymorphonuclear leukocyte in the pathogenesis of Rhizopus oryzae infection. An adequate response of normal polymorphonuclear leukocytes inhibits the development of mucormycosis while the metabolic alterations associated with cortisone administration, although insufficient to induce widespread infection, clearly enhance the host's susceptibility to this fungus.*

SUMMARY

Cortisone administered to rabbits for 4-23 days preceding inoculation with the fungus Rhizopus oryzae slightly decreases host resistance to this

*The technical assistance of Miss Hillma Gheesling and Miss Elaine Schubert is gratefully acknowledged.

agent but does not lead to widespread infection. An inflammatory focus produced by croton oil in the nose and lung of normal rabbits and subsequently inoculated with Rhizopus oryzae inhibits the proliferation of the fungus.

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GROWTH OF GUINEA PIG TOOTH GERMS SIMULTANEOUSLY TRANSPLANTED WITH A HUMAN GLIOBLASTOMA MULTIFORME**†

INTRODUCTION

The demands for energy-rich metabolites by particular organs during prenatal growth and development are so great that the entire resources of the embryo are mobilized at specific times.[12] There is an increased rate of activity of the major metabolic pathways during embryonic development on demand by particular sites. The product or result of this particular activity is growth of the organ or organs in question, and the requirements of the anlagen of teeth during the formation of the pre-enamel and pre-dentine matrices are no different.[4, 16] During such times any environmental insult resulting in a lack of available metabolites may produce congenital anomalies in those teeth upon which the embryo is at that time concentrating.[5, 13]

Tumors consume large amounts of energy for they have a higher aerobic and anaerobic glycolysis than most normal tissues.[2, 8, 19] Warburg[18] states that tumor cells utilize the energy of carbohydrate breakdown from a synthesis of cellular proteins to a larger extent than most normal tissues.

In transplanting spontaneous mammary tumors with normal tissues of different strains of mice, Browning[1] observed there was for a time a stimulation of the normal tissue growth. As the alien tumor cells began to invade the growing embryonic homotransplants, focal concentrations of lymphocytes appeared which aided growth of the embryonic tissues by destroying the tumor cells. In studying the interaction between transplanted normal and malignant suspensions of cells, Schneyer[17] did not obtain results similar to Browning. Oker-Blom and Alfthan[15] had sporadic success with normal and neoplastic tissues dually transplanted to the chorio-allantoic membrane. It was observed by Malmgren's *et al.*[14] that inter-strain tumor transplants failed to survive in tumors of a different genetic derivation, but when they

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** Presented in part at the 34th Annual Meeting of the International Association of Dental Research, St. Louis, Missouri, March 22-25, 1956.

† Research supported by a USPHS grant from the National Institutes of Health, National Institute of Dental Research, Department of Health, Education and Welfare, Bethesda, Maryland.

Received for publication April 16, 1956.

were of the same genetic derivation, the tumor implants did survive. Grobstein and Younger[11] concluded that tissue from two different species of animals may be grown simultaneously in flask cultures without apparent incompatability.

There have been no reports on the intra-ocular transplantation of a human neoplasm with embryonic tissues (tooth germs) in experimental animals.

METHODS

The techniques of tumor[9] and of tooth germ transplantation[8] have been described in detail.

Several other transplantable tumors were employed in pilot studies with tooth germs, but these neoplasms outgrew and destroyed the tooth germ transfers. These tumors were Sarcoma 37, MT 65, and Sarcoma 180.

The human tumor used in these experiments was a glioblastoma multiforme and was obtained from a patient in the hospital operating room. For several years it has been carried serially in our laboratories in experimental animals. Its behavior upon transplantation to the eyes and/or brains of rabbits, rats, mice, and guinea pigs has been extensively studied.[10] After a latent period of 20-30 days in the eyes or brains of the above-mentioned experimental animals, its growth is continuous and expansive. The tumor cells that survive transplantation are spongioblasts along with more primitive glial cells and giant cells. Before transplantation, necrotic tumor tissue and normal brain tissue were discarded and only fresh viable tumor in pieces of 1-1.5 mm. square were used in these studies.

Tooth germs were obtained from 25-35-day-old guinea pig embryos. The embryo or embryos of a single pregnant animal furnished the tooth germs for each series. On this basis, 27 different groups or series of varying numbers of young adult guinea pigs received transplants.

Each series was subdivided into three groups of animals. One group received tumor tissue alone, a second group received tooth germs alone, and the third group received tooth germs with tumor tissue. In this latter group, a total of 235 young adults of both sexes received transplants of tumor and tooth germs. A total of 112 animals received anterior chamber transplants of tumor tissue and 135 animals received transplants of tooth germs.

Tumor and tooth germs were allowed to grow together for varying periods from 35-350 days. Control animals, bearing only tooth germ transplants, were kept for periods varying from 35-125 days. Hosts with tumor tissue only were not sacrificed until the corneas were about to rupture. This usually occurred from 75-90 days following transplantation.

Moribund hosts were sacrificed at once and transplanted tissues, when present, recovered. Animals that became sick, developed eye infections, or failed to show evidences of the growth of transplants were discarded. These are listed in Table A but are deleted from a comparison of the results between tooth germ with tumor tissue and tooth germ alone.

Eyes were checked periodically and the quality of each transplant's growth recorded and graded. Excellent growths were those that almost completely filled the anterior

chamber by 60 days, did not regress, and retained the morphology of the teeth. Transplants were graded from this level down to poor and negative growths. Those that grew for a time and then resorbed were considered poor. When tissues could not be observed in the anterior chambers following transplantation or they resorbed completely, these were considered negative growths. The growth of transplants was recorded in the following manner: ++++ excellent, +++ good, ++ fair, + poor, and — negative.

Recovered transplants were formalin-fixed and decalcified when necessary. Tissues were sectioned at 5-7 μ and stained with hemotoxylin and eosin for histological study.

TABLE A. QUALITY AND NUMBER OF TAKES*

Growth	*Tooth germ with tumor*	*Tooth germ without tumor*	*Totals*
++++	63 (44.7)	9 (27.3)	72
+++	42 (43.4)	28 (26.6)	70
++	28 (38.5)	24 (23.5)	62
+	20 (28.5)	26 (17.5)	46
—	35 (32.9)	18 (20.1)	53
Totals	188	115	303

Expected numbers written in brackets.
X^2 with 4 degrees of freedom = 34.45.
$P < 0.001$.

RESULTS

The primary purpose of these studies was to determine the effect of this tumor tissue on growth and development of tooth germs when these tissues were transplanted together to the anterior eye chambers of guinea pigs.

When transplanted alone in the present experiments, this glioblastoma multiforme grew very slowly at first, but by 30-40 days it began to grow more rapidly. By 75-90 days its expansive growth usually ruptured the corneas. Figure 1 shows the tumor just rupturing the cornea 85 days following transplantation in one of the control series of the present experiments. Figure 6 shows a histological section of the tumor as seen grossly in Figure 1. This can be compared with Figure 5, which shows a histological section of the original tumor used for these experiments upon recovery from the brain of a guinea pig 70 days after transplantation.

The following biological events took place when tooth germs were transplanted alone in the present experiments. After a latent period of 4 or 5

* Discarded animals not included in above table: 25 sick, died, or lost—with tumor and tooth germ; 22 eye infections—with tumor and tooth germ; 11 sick, died, or lost —with tooth germ; 9 eye infections—with tooth germ.

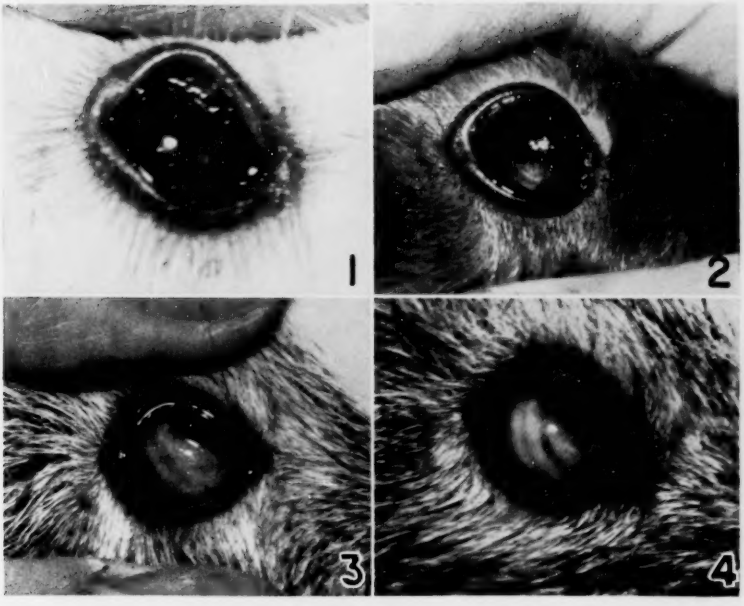

FIG. 1. Anterior chamber transplant of tumor used as control for a series in the present experiments after 85 days. It is just rupturing the cornea.

FIG. 2. Anterior chamber transplant of tooth germ from a 30-day guinea pig embryo 65 days after transplantation. Control for series 17 in the present experiments.

FIG. 3. Anterior chamber transplant of tumor and tooth germ from 30-day guinea pig embryo 65 days after transplantation. Same series as above.

FIG. 4. Represents Figure 3, 125 days after transplantation.

Key to Symbols

D dentin
E enamel
G giant cells
I pigmented cells of iris
O odontoblasts
P pulp
T tumor
Bv blood vessels
OD osteodentin

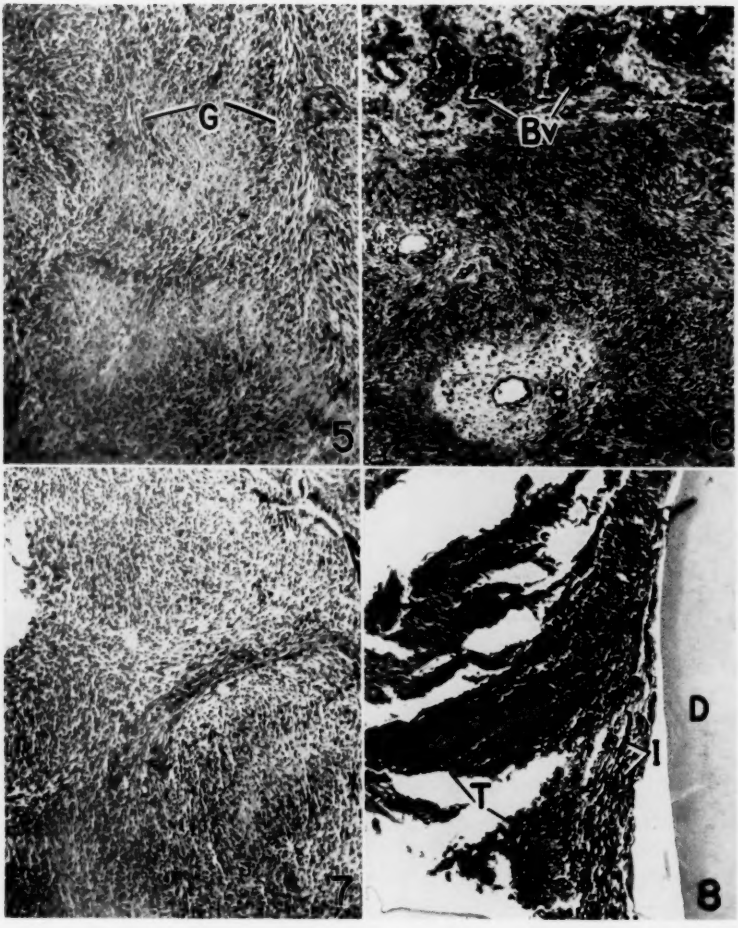

FIG. 5. Histological section of the original tumor used for these experiments recovered after 70 days from the brain of a guinea pig. Original magnification x165.

FIG. 6. Histological section of the tumor shown *in situ* in Figure 1, after recovery from the host. Original magnification x165.

FIG. 7. Histological section of tumor 335 days after it was transplanted with a tooth germ. Tissue shown is approximately the entire amount of tumor recovered. Original magnification x165.

FIG. 8. Shows degenerating tumor tissue in contact with tooth transplant 76 days after transplantation. Original magnification x165.

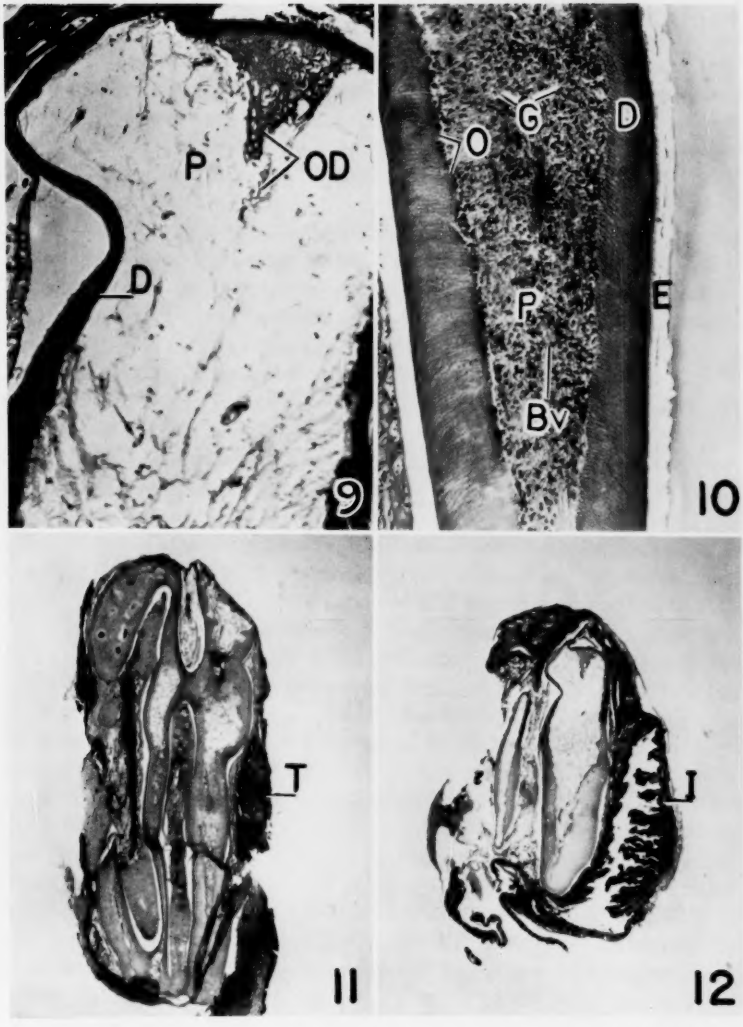

FIG. 9. Shows pulpal area of transplanted control tooth germ 65 days after transplantation. Original magnification x165.

FIG. 10. Shows pupal area of tooth germ transplanted with tumor 65 days after transplantation. Original magnification x165.

FIG. 11. Entire tooth germ transplant partially shown in Figure 10, and grown with tumor, for comparison with Figure 12.

FIG. 12. Represents tooth germ grown alone, partially shown in Figure 9.

Original magnification of Figures 11 and 12, x19.

days, the transplants became attached to the irises by connective tissue cells and were then vascularized. Differentiation, growth, and development were usually rapid and continuous until mature calcified tooth structures were formed. After this, there was a gradual decrease in vascularity. The pulpal areas of such transplants also became less vascular and in addition their cellularity diminished. An osteoid revision followed these changes. It began within the pulps and extended to or involved adjacent dentine so that its customary tubular appearance was finally lost. The rate and extent of this osteoid revision varied, but usually it was initiated from 30-60 days following transplantation and has been extensively reported.[3] Figure 2 shows a transplant of a tooth germ in place 65 days after it was placed in the anterior chamber. Histological sections are shown in Figures 9 and 12, and here the characteristic osteoid revision, avascularity, and acellularity are demonstrated.

The major observation in the present experiments was on enhancement of growth and development of a significant number of tooth germ transplants that were transplanted with the tumor. When both tumor and tooth germs survived transplantation together and maintained a contiguous relationship, development of the teeth took precedence over the tumor. This resulted in the formation of greater amounts of enamel and dentine. At such times, the tumor tissue also grew for a while, but by 100-125 days it had usually disappeared entirely. When transplanted with tooth germs, the tumor never seemed capable of growing expansively enough to rupture the corneas although the developing tooth sometimes did. Figure 7 shows a histological section of tumor taken from an anterior chamber after 335 days with a developing tooth. This tooth germ transplant filled the anterior chamber by 90 days and maintained itself with an active blood supply for the ensuing period. The accompanying tumor after growing for awhile, resorbed and only a remnant about 2 mm. square was recovered. Continued growth of a tooth transplant in the presence of tumor tissue is demonstrated by Figures 3 and 4. Figure 3 shows the transplanted tissues after 65 days and Figure 4 indicates their condition after 125 days. These were from the same series or group from which Figure 2 was taken. In both instances, 30-day guinea pig embryonic litter mates supplied the tooth germs which were precursors of the third mandibular cheek tooth. Comparative difference in size in a control transplant and one transplanted with tumor tissue are shown in Figures 11 and 12. Figures 9 and 10 show the histological differences in the pulpal areas. Figure 8 shows a small bit of degenerated tumor tissue beside a tooth transplant after 76 days.

Pulpal areas of these transplants never appeared to be invaded by tumor cells when the tooth germ transplants remained intact and were not dam-

aged during transplantation. At times giant cells that closely resembled gitter cells were found within the pulp. Pulpal areas failed to become avascular and acellular as they did in control experiments with tooth germs. Pulpal cells remained young-appearing and blood vessels were more numerous than in control transplants with tooth germs. Odontoblasts remained in their normal position along the periphery of the pulp, and they did not disappear from the pulp as early as they did in control transplants.

Cells of the inner enamel epithelium, the ameloblasts, appeared to remain active and closely packed together for longer periods than those in the control animals. This continued activity resulted in the formation of more enamel than was found in the controls.

DISCUSSION

The ability of tooth germ transplants to survive and outgrow this tumor when both were simultaneously transplanted to a location where this tumor was known to survive and grow is in sharp contrast to the events taking place when certain faster growing tumors were used in pilot studies. The extent of this enhanced growth in a significant number of instances was greater than the expected growth and compared favorably with growth obtained in guinea pig female castrates.[7]

The biological behavior of the tooth germs and of this human neoplasm when transplanted together to the same location is of interest. Apparently growth potentialities of organ anlagen such as the teeth can be increased by transplanting them with certain transplantable neoplasms of approximately equal growth rates. The lack of a significant lymphocytic response by the hosts indicated that these tissues were compatible when transplanted together. Over the long periods of the present experiments, the tumor cells, after stimulating endothelial cells to proliferate and form capillaries or vascular channels, may never have had an opportunity to use these vessels entirely for their own growth and development. Furthermore, the subsequent rapid growth of the tooth germs apparently crowded out the tumor, leaving no space for it to grow.

It is difficult to compare the results obtained in these studies with the investigations of others because little has been done in this particular area of experimental biology. The nearest approach to a study of this type was the work of Browning,[1] where with different strains of mice he transferred alien tumors with homotransplants of embryonic tissues. When a tumor had the power of autonomy, he observed that growth of the embryonic tissue transplants was enhanced. The main point was that growth of embryonic tissues continued until that state of development normally attained

at birth was reached. In the present experiments, however, growth of tooth germ transplants was such that they often attained and sometimes exceeded the size of their *in situ* counterparts in adult hosts. Perhaps his (Browning's) results might have been different if it had been possible to transplant whole organ anlagen with tumor tissue such as was done with tooth germs in the present experiments. It is quite conceivable that tooth germs as organs are organized with a greater determination for growth than mere groups of tumor cells. This seemed especially true since both tumor and tooth germ transplants were initially about the same size and both had similar growth rates as indicated in these experiments.

It has been noted that congenital anomalies of teeth are the results, directly or indirectly, of metabolic insults occurring during embryonic growth.[5, 18] Ameloblasts, the highly specialized cells of the enamel organ, are greatly affected by environmental changes.[10] The effect of prenatal influences on growth and development of the teeth has been sparsely investigated. Some information is available concerning factors or conditions that may retard or interfere with differentiation, growth, and development of the anlagen of the teeth, but there is little basic information about factors that are capable of augmenting their development. It is largely for this reason that this communication and a previous one[7] may be of interest.

Basic metabolic machinery is a common property of all mammalian cells, and interruption of metabolic processes is potentially equal for all cells.[12] However, tissues may vary in their responses to changes or alterations in the availability of metabolites. Such responses are an indication of differences in their dependence or vulnerability in a direct or indirect manner to oxygen or glucose deprivation. While most observations relative to growth and development of teeth have been on a negative side, very little information is available where environmental conditions are such that growth is enhanced instead of being interfered with. In the present experiments, conditions favored the enhancement of growth of the tooth germ transplants.

SUMMARY

When these human tumor and guinea pig tooth germs were transplanted together to the anterior eye chambers of guinea pigs, growth of the tooth germs and not the tumor was enhanced if these tissues maintained a continguous relationship. Control transplants of tooth germs never attained the size of their counterparts which were transplanted with this human glioblastoma multiforme. Tumor tissue capable of rupturing the anterior chambers by expansive growth from 75-90 days after transplantation, when transplanted alone, did not do so when transplanted with tooth germs. At

times, however, the continued growth of the tooth germ transplants caused a rupture of the corneas, a fact which did not ordinarily happen with these single transplants. Tumor tissue survived for varying periods but usually it was finally resorbed when in the presence of a transplanted tooth germ. Enhancement of growth in these experiments was comparable to the augmented growth obtained in guinea pig female castrates.

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PROPERTIES AND BIOLOGICAL SIGNIFICANCE OF CHOLINE ACETYLASE**

INTRODUCTION

A. HISTORICAL BACKGROUND

In recent years the rôle of acetate in intermediary metabolism has been one of the most actively studied fields in biochemistry. The first indication of its physiological importance goes back to the investigations of Knoop.[84] In 1904 he concluded that fatty acids were oxidized stepwise with the formation of a two-carbon compound, presumably acetic acid, and postulated his well-known β-oxidation theory. This concept was further supported by Dakin,[19] who found that the presumed intermediates in this oxidation—the α-β unsaturated acid, the β-hydroxy, and the β-keto acid—gave similar yields of acetoacetic acid.

For a long time there was little further progress made in elucidating the rôle of acetic acid in intermediary metabolism. In the 1930's Schoenheimer and Rittenberg performed their early experiments with deuterium.[80, 72, 73] In animals fed D_2O, the fatty acids formed had close to half the isotope concentration of the body water, thus indicating that about half of the fatty acid hydrogen is derived from the water. Schoenheimer, therefore, suggested that the fatty acids are formed by the coupling of small units.[72]

Shortly after this, Bernhard[8] fed deuterio-labeled acetate together with sulfanilamide to rabbits and rats and recovered deuterioacetylsulfanilamide in the urine. p-Amino benzoic acid was also found conjugated with the labeled acetate in the rabbit. The experiments of Rittenberg and Bloch[68] in which $CD_3C^{13}OOH$ was fed to rats, resulting in fatty acids with alternate $C^{13}H_2$ and CD_2 methylene groups and a terminal $C^{13}OOH$, gave further

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** This work was supported by the Division of Research Grants and Fellowships of the National Institutes of Health, H-740, United States Public Health Service.

Received for publication April 25, 1956.

evidence for the incorporation into the fatty acid of the acetate 2-carbon unit as a whole.

All the above experiments were performed with whole animals or organs. In 1938 Klein and Harris[86] observed that rabbit liver slices acetylate sulfanilamide and p-aminobenzoic acid *in vitro* in the presence of added acetate. This was the first demonstration of acetylation *in vitro*. However, since the reaction was an aerobic process and occurred only in the intact liver cell, it did not indicate the mechanism of acetylation because the energy of respiration may be used for a great number of reactions.

In 1943 Nachmansohn and Machado[57] extracted a cell-free system from brain and electric tissue in which acetylation of choline was observed if ATP was present. This was the first biological acetylation achieved in a soluble system; it demonstrated that ATP provides the energy for acetate activation in biological acetylation. In view of Nachmansohn's result, Lipmann[37] tested, in 1945, whether ATP is also the energy source in the acetylation of sulfanilamide by pigeon liver extracts. His positive results further emphasized the general nature of this reaction.

An important further step was the discovery and purification of a cofactor involved in acetylation. In 1943 it was observed by Nachmansohn *et al.*[56] that the choline acetylating system is rapidly inactivated on dialysis; this suggested the existence of a coenzyme. In 1945 Nachmansohn and Berman[55] prepared a partially purified preparation of a coenzyme from "Kochsaft" of liver, heart, and brain, which reactivated a dialyzed choline acetylating system prepared from acetone dried powder of rat brain. The existence of a coenzyme of choline acetylase was also independently demonstrated by Lipton.[48]

At about the same time, Lipmann[37] found that pigeon liver sulfanilamide acetylation also required a cofactor. When the two systems, and the action of the coenzyme were studied, it became apparent that the cofactor must be the same. Acetylations on oxygen and nitrogen atoms, therefore, may have a basically similar pathway since both require ATP and apparently the same coenzyme. Lipmann proposed that the coenzyme be called Coenzyme A (for acetylation).

The even greater generality of the reaction and the importance of the coenzyme in acylation reactions were soon recognized. An important contribution towards the understanding of the mechanism of acetylation and its relation to fatty acid synthesis was made by Stadtman and Barker[82] in 1949. They prepared a cell-free enzyme system from *Clostridium kluyveri* which

catalyzed the anaerobic synthesis of 4 and 6 carbon fatty acids from ethanol. In their system, ethanol and butyrate gave rise to acetyl phosphate by aerobic oxidation,[83] and, conversely, acetyl phosphate and acetate were reduced to butyrate.[84] A cofactor from the dialysate, which later proved to be CoA, was necessary.

Acetyl phosphate had been considered as the probable "active acetate"[85] and as an intermediate in fatty acid synthesis.[86] However, it proved to be active only in some bacterial systems and not at all in animals or yeast.

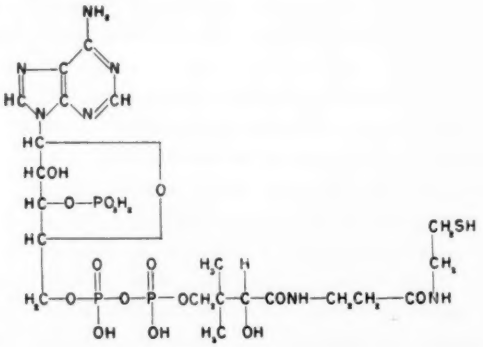
Stern and Ochoa[84] demonstrated that citrate was readily formed from acetate and oxalacetate in the presence of pigeon liver fractions, CoA, ATP, and Mg^{++}. These investigations, as well as experiments by Soodak and Lipmann,[78] further suggested that since the addition of oxalacetate markedly decreases the formation of acetoacetate and acetyl sulfanilamide, the condensation is, in all three cases, with the same acetyl derivative. These and many other observations indicated that acetate and CoA were involved in a great variety of pathways of intermediary metabolism. Table 1 summarizes a great number of acetyl donor systems to, and acceptor systems from, acetyl CoA which have been identified in the last few years.

As is indicated in this summary, a number of other acyl groups besides the acetyl group take part in this reaction. These include the various intermediates in the oxidation of fatty acids, which are all CoA derivatives[45, 47]; propionyl, butyryl, and other acylated CoA compounds from which the CoA can be transferred to the acetate group with Stadtman's[80] transphorase; succinyl CoA which is involved in the citric acid cycle[23]; and benzoyl and other acyl CoA's which can be conjugated with glycine.[10, 72]

B. COENZYME A

1. *Structure.* In 1947 Lipmann *et al.*[39] demonstrated that CoA comprised a pantothenic acid moiety linked to a nucleotide by a phosphate bond. Snell *et al.*[77] reported that in their bacterial growth substance, the *Lactobacillus bulgaricus* factor (L.B.F.), the β-alanine of the pantothenic acid is bound to β-mercaptoethanolamine, giving N-pantothenyl-β-mercaptoethanolamine. This substance could be obtained from CoA by treatment with intestinal phosphatase,[47] and is the pantetheine half of the CoA molecule. Baddiley and Thain[1, 2] studied the attachment of the phosphate groups to the pantotheine and found this half of the molecule to be the 4′phosphopantetheine. By specific enzyme action, the second phosphate was found to be in pyrophosphate linkage with the first, while the third phosphate group was placed on

the 3′ position of the ribose.[75] The structure was confirmed by synthesis[65, 66] and found to be as follows:



The active group of the acetylated coenzyme was elucidated by Lynen *et al.*[65] early in 1951. They showed that free CoA gives a positive nitroprusside test, is inhibited by iodoacetate and gives other sulfhydryl reactions. Once acetylated, on the other hand, it showed none of the above reactions. They concluded that coenzyme A is acetylated on the sulfhydryl group.

2. *Energy content of acetyl CoA.* The acetyl sulfhydryl bond was found to be quite labile. The ΔF° of acetyl CoA was determined to be 10,000 to 12,000 calories when calculated from different reactions. Stern *et al.*[95] used citrate formation from acetyl CoA and oxalacetate coupled with malate oxidation by DPN to oxalacetate.

Stadtman[79] measured the equilibrium of the phosphotransacetylase reaction: Acetylphosphate + CoA $\rightleftharpoons$ acetyl CoA + phosphate and obtained a value very close to that of Stern *et al.* It was therefore established that the acetyl coenzyme A molecule is a high energy compound representing a new type of biologically active energy-rich compound.

The source of energy for this acetylation intermediate in animal cells and yeast is ATP, as discussed above. Acetylphosphate is apparently utilized by many bacteria, as is indicated by the equilibrium catalyzed by phosphotransacetylase[80]:

$$\text{Acetylphosphate} + \text{CoA} \rightleftharpoons \text{acetyl CoA} + \text{P (inorg.)}$$

It has also been found that acetylphosphate is synthesized from acetate and ATP by the enzyme acetokinase,[70] thus interlocking the three high energy substances. The various acyl CoA's can also be interconverted.[80]

TABLE 1. SUMMARY OF SYSTEMS FORMING AND UTILIZING ACETYL CoA

Donor systems			*Acceptor systems*			
Ref. no.	*Substrate*	*+ AcCoA-forming enzyme*	*AcCoA-utilizing enzyme*	*Product*		*Ref. no.*
21	Acetyl phosphate	phosphotransacetylase	Choline acetylase	Acetyl	choline	29
9, 22	Acetate + ATP	acetylkinase acetate activating enzyme	Pigeon liver extract	"	sulfanilamide	10, 30
			" " "	"	amines, generally aromatic	31
23	Pyruvate + DPN Lipoic acid, Cocarb, Magnesium	pyruvic oxidase	" " "	"	histamine	32
			" " "	"	hydroxylamine	33
24	Acetoacetate + Succinyl CoA	activation and cleavage enzyme	" " "	"	acetoacetate	34
25	Acetaldehyde	aldehyde dehydrogenase	Different pigeon liver fractions	"	glucosamine galactosamine	35
26, 27	Fatty acids	cleavage enzyme β keto thiolase	Glycine N-acylase C kluyverii extracts	Acyl	amino acids	36, 37, 38
28	Acetate + Propionyl CoA, Butyryl CoA, etc.	CoA transphorase	Condensing enzyme		citrate	39
			Activating enzyme		fatty acids	40, 41, 42
			CoA transphorase + propionate, butyrate, etc.		propionyl butyryl CoA	28
			S. cerevisiae cells Rat liver slices		steroids	43
			Imidazoleacetylase	Acetyl	imidazole	44

Another major source of energy for acetylation reactions is the energy derived from oxidative decarboxylation. Schwett and Cheslock[74] reported that in the absence of ATP and acetate, pyruvic acid and the pyruvic oxidase system acetylated sulfanilamide faster than the ATP-acetate system. Burton and Stadtman[14] suggest that DPN catalyzed acetaldehyde oxidation can also be an energy source for acetyl CoA formation.

3. *Chemical mechanism of the condensation reaction.* The mechanism by which the activated acetyl group reacts with the acceptor compound apparently also varies. In most cases it is the carboxyl-carbon of the acyl group which is activated by forming the CoA thiolester. This carbon is bound to the acceptor in the numerous N- and O-acylations. The acceptor atoms are relatively negative, while the relatively positive character of the carbonyl carbon is increased by bonding with the sulfur on the sulfhydryl group of the CoA. However, when carbon-carbon condensations are considered, it is apparent that the methyl carbon is activated and combined with a strongly positive carbon on the acceptor.[44]

4. *Preparation of CoA and acetyl CoA.* CoA has been purified from 50 to 90 per cent in three different laboratories with various yields.[24, 9, 88] All three groups of investigators used primarily the techniques of adsorption on and elution from charcoal and resins.

Acetyl CoA was first prepared synthetically by Wilson[100] with thioacetic acid. Other acyl CoA's were prepared from the corresponding thiol acids.[80] A higher yielding and more convenient method involving the desired anhydride was later devised by Simon and Shemin.[78]

C. ENZYMATIC MECHANISM OF FORMATION OF ACETYL COA FROM ATP AND ACETATE

Lipmann *et al.*[78] reported the formation of pyrophosphate when acetyl CoA was formed from ATP, acetate, and CoA. Lipmann and Lynen and their associates[80] suggested a three-step mechanism of acetyl CoA formation involving an AMP-enzyme intermediate. Recent evidence indicates, however, that a different mechanism must be involved. Boyer *et al.*[9] found that an anhydride linkage is an intermediate of acetyl CoA formation. The AMP acetate anhydride was synthesized by Berg[4] and found to be an intermediate in this reaction. He suggests the following mechanism:

1. ATP + acetate $\rightleftharpoons$ adenyl-acetate + P-P
2. Adenyl-acetate + CoA $\rightleftharpoons$ acetyl CoA + adenylic acid

The ATP-acetate system, but not the phosphotransacetylase system, was found in *Rhodospirillum rubrum* by Eisenberg.[30] In studying the choline

acetylase system of *Lactobacillus plantarum,* Girvin and Stevenson[21] also used ATP-acetate as the acetyl donor; they did not mention whether acetyl-phosphate was tried as an acetyl source. The ATP-acetate system has also been found in plants by Millerd and Bonner.[48]

Thiolacetate can replace ATP-acetate in the formation of acetyl CoA using acetylkinase, the acetate activating system, from pigeon liver or rabbit brain.[49] In a pigeon liver extract, prepared by ammonium sulfate fractionation, thiolacetate was 25 per cent as active as ATP-acetate when the most active fraction of each was used. Presumably the reaction is:

$$CH_3C(=O)SH + HSCoA \rightleftharpoons H_2S + Ac\text{-}SCoA$$

This reaction may be similar to Stadtman's CoA transphorase system discussed above, or another enzyme and an acetyl-enzyme intermediate may be involved. Brady and Stadtman[10] have found the reverse of this reaction to be catalyzed by a special enzyme which they call H_2S-thioltransacetylase. They consider this reaction to be analogous to a series of other thioltransacetylation reactions, although catalyzed by a separate enzyme. The reaction is significant in that it indicates the high reactivity of the thiol-acyl bond.

D. BIOLOGICAL SIGNIFICANCE OF ACETYLCHOLINE FORMATION

The cycle of acetylcholine formation, utilization, and breakdown functions primarily as part of the process of nerve impulse conduction. The energy source of the electric currents which propagate nerve impulses are ionic concentration gradients across the nerve membrane. Sodium ions are highly concentrated in the outside fluid and low in the interior. The reverse is true for potassium ions. As a stimulus is carried along a nerve, there occurs a sudden influx of sodium ions in the active region and an equivalent number of potassium ions flow to the outside. These ion movements are the carriers of the electric currents propagating the impulse. A change in the permeability of the membrane is necessary to make the concentration gradient effective and to permit the entrance of sodium ions. This alteration is considered to be the effect of acetylcholine acting on certain proteins of the membrane. The possible changes in protein configuration and the evidence for it will be discussed in greater detail later in this article.

Acetylcholine is stored in the membrane of the nerve bound to a protein; as the electrical impulse reaches the area, it is released, acts on a membrane protein and is very rapidly destroyed by acetylcholinesterase. The protein

returns to its resting condition and the barrier for ion movements is re-established. This immediate recovery makes it possible for the next impulse to pass. The choline acetylase resynthesizes acetylcholine in a subsequent recovery phase from acetyl CoA and choline which, as has been discussed before, requires ATP. A thorough discussion of the rôle of acetylcholine in nerve function may be found in review articles by Nachmansohn.[50, 51, 52, 59]

The system catalyzing acetylcholine synthesis has been identified in numerous vertebrate, invertebrate, and even bacterial extracts. It has been found in all conductive tissues studied, such as, among vertebrates, rat or rabbit brain,[57, 65, 91] nerve tissue,[55] electric organ of eel,[67] striated muscle,[54] and heart muscle.[12] It is present in motor and sensory nerve fibers[59]; it was found in Squid head ganglia,[58] abdominal chain of the lobster,[53] and in an annelid, flatworm, coelenterate,[64] and even in a very motile protozoan, the trypanosome.[18] It seems also to be present in red blood corpuscles, which also have a high acetylcholinesterase concentration,[25] in spleen,[16] which breaks down red blood cells, human placenta,[19] and in the bacterium *Lactobacillus plantarum*.[92] Its function in the latter organism is unknown, although acetylcholine synthesis has been found to be correlated with growth and acid production there.[21]

E. ENZYMATIC SYNTHESIS OF ACETYLCHOLINE

When Nachmansohn and Machado prepared their cell-free choline acetylating system,[57] the detailed mechanism was not yet clear. The acetylation of choline is now known to comprise two enzymatic steps, the first being the formation of acetyl-S-CoA from any donor system. The term "choline acetylase" has been redefined to refer to the specific acceptor enzyme catalyzing choline bonding with the acetyl group. The reaction is:

$$CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{—S-CoA} + HO\text{-}CH_2\text{-}CH_2\text{-}\overset{CH_3(+)}{\overset{|}{N}}(CH_3)_2 \rightarrow CH_3\text{-}\overset{O}{\overset{\|}{C}}\text{--O-}CH_2\text{-}CH_2\text{-}\overset{CH_3(+)}{\overset{|}{N}}(CH_3)_2 + HS\text{-CoA}$$

Since acetyl-S-CoA is now easily prepared synthetically, the choline acetylase reaction could be studied apart from other enzymes. The results of specificity and inhibitor experiments on this reaction could now be compared with those obtained for the hydrolysis of the ester by acetylcholinesterase[59, 101, 102, 105] and similarities and differences of enzyme-substrate affinity could be investigated.

An even more compelling reason for using acetyl CoA instead of free CoA and an acetyl donor system was the fact that this made it possible to study the sulfhydryl groups on the enzyme without interference from the coenzyme SH. Compounds known to react with sulfhydryl groups could then only affect the enzyme.

In view of its biological importance more information about the characteristics and mechanisms of action of choline acetylase appears desirable. Some recent studies on these problems are described below.

METHODS

A. ENZYMES

1. *Choline acetylase separation.* Acetone powder prepared from Squid head ganglia was extracted with .02 M phosphate buffer at pH 7.4. Ammonium sulfate was added to the supernatant to a concentration of 25 grams per 100 ml. solution, and the precipitate was centrifuged. It was then dissolved in phosphate buffer, dialyzed, and treated with protamine sulfate. The precipitate formed was centrifuged and discarded and the supernatant was reprecipitated with ammonium sulfate. The fraction obtained between the ammonium sulfate concentrations of 16 to 28 grams in 100 ml. of solution was adsorbed on calcium phosphate gel at pH 6.2. The eluate obtained with 0.2 M phosphate buffer at pH 8.2 contained most of the activity and was again precipitated with ammonium sulfate at 30 per cent. A more detailed description of the purification may be found elsewhere.[7]

Although the enzyme was purified only tenfold, giving a specific activity between 30 and 80 uM of acetylcholine formed per mg. protein per hour (depending on the activity of the initial powder), it was free from most of the interference by acetyl CoA deacylase. The residual deacylase activity was at least less than 10 per cent (and often less than 5 per cent) of the choline acetylase activity after one hour's incubation time. The enzyme lost one-third of its activity in one and one-half months when stored in the deep freeze in the presence of 1 uM per ml. EDTA.

2. *Transacetylase.* Potassium phosphate buffer extract of lyophilized *E. coli* was used as a source of phosphotransacetylase[47] to form acetyl CoA enzymatically during the course of the reaction. The extract can be partially purified by precipitating it with 45 grams of ammonium sulfate added for each 100 ml. of extract.

B. PREPARATION OF ACYLATED COA DERIVATIVES

Acylated forms of CoA may be prepared by a modification of the method of Simon and Shemin.[78] This involves adding acetic anhydride to a solution of CoA and sodium bicarbonate at a pH of 7.5 to 8.0. It may be noted that most of the CO_2 must be removed by acidification of the solution before the pH is adjusted to 6.8 and the acetyl CoA is stored. Otherwise the gas is slowly released on standing and the solution becomes more alkaline, thereby splitting the acetyl CoA.

An excess of acid anhydride of more than about one-third should also be avoided; otherwise preparations are obtained which are less active substrates for acylation reactions. They show more than one equivalent of acyl groups for each equivalent of

SH as assayed by the hydroxamic acid and nitroprusside methods discussed below. Apparently another group on the CoA molecule is also acylated, possibly the amino group of the adenine; this more highly acylated compound is either inactive or may even be inhibitory.

The various acyl derivatives of CoA exhibit different degrees of stability both to alkali and upon incubation at 31° and pH 7. Acetyl, butyryl, propionyl, and succinyl CoA's were prepared and tested for ester formation in the choline acetylase system. Benzoyl CoA was kindly supplied by Dr. John Taggart.[71]

Acetyl pantetheine was prepared from pantetheine in a manner similar to that of acetyl CoA. The pantetheine was obtained by reducing pantethine (kindly sent by Dr. E. E. Snell) with 10 equivalents of sodium borohydride. The product obtained was tested for free sulfhydryl groups with the nitroprusside reaction. The pantetheine formed was 100 per cent acetylated, as assayed by the hydroxamic acid test.

Coenzyme A can be obtained commercially from Pabst; 1 mg. of this product was found to contain 1 μM of sulfhydryl groups. 97 to 100 per cent of these are acetylated with acetic anhydride, indicating that this reaction goes to completion.

Using 10 times the usual choline acetylase concentration, and one-tenth the usual acetyl CoA concentration, only .84 to .88 μM of acetylcholine were formed for each μM of acetylated SH added. The reaction was complete after five minutes, and there was no increase in acetylcholine formation, even with an incubation time up to two hours. The value obtained is in good agreement with Stadtman and Kornberg's finding[80] that each mg. of Pabst CoA contains .85 μM of CoA on the basis of arsenolysis, phosphorus, and adenine determinations. The rest of the sulfhydryl groups determined are probably due to the presence of glutathione which is acetylated but, as will be shown later, not utilized to form acetylcholine. Thus, there is about .1 to .15 μM of glutathione in each mg. of the commercial preparation. In addition, it contains about 30 per cent of other impurities.

Acetylphosphate, used for experiments in which acetyl CoA is regenerated from CoA by phosphotransacetylase during the course of the reaction, may be prepared from isopropenyl acetate and phosphoric acid by the method of Stadtman and Lipmann.[80]

C. ASSAY METHODS

The alkaline hydroxamic acid method of Hestrin[27] was used to assay choline acetylase activity during purification of the enzyme.

The choline acetylase catalyzed reaction involves the disappearance of two substrates, choline and acetyl CoA, and the appearance of two products, acetylcholine and CoA. Assay methods may, therefore, be based on any one of these four compounds. In earlier experiments the disappearance of the acetylthiolester was determined by the hydroxamic acid test in neutral solution.[41] Acetyl-S-CoA is labile enough so that it can be readily converted to acethydroxamic acid, while acetylcholine is stable under these conditions. This reaction was calibrated with acetylthiocholine.

It was later found that the nitroprusside test for the sulfhydryl group of the liberated CoA was about six times as sensitive as the above reaction; therefore the hydroxamic acid method was no longer routinely used to assay enzyme activity in the isolated choline acetylase system.

The nitroprusside test, as used for most of the assays, is a modification of the Grunert-Phillips quantitative method.[25] The details of the method have been described in an earlier paper.[7] It involves the formation of a red color presumably due to the reduction of the Fe+++ in the sodium nitroprusside to Fe++ by the sulfhydryl groups. This reduction takes place at pH 10, and in the presence of sodium cyanide, which has been reported to increase the stability of the color.[26] Under the conditions used, 5×10^{-3} μM of sulfhydryl groups per ml. would give an optical density of .018 at $\lambda = 520$, and can thus be readily estimated. Applying a correction for the breakdown of residual acyl CoA at alkaline pH and reading each assay exactly 30 seconds after the color is first developed, the readings are reproducible within 5 per cent.

When the activating effect of cysteine was established and this compound was added to the reaction mixture, the nitroprusside method was no longer feasible. The determination of acetylcholine formed by bioassay had to be resorted to.[15] This method is even more sensitive than the nitroprusside test (2×10^{-4} μM of acetylcholine can be estimated) but it is much more time consuming.

Protein concentrations were determined primarily by a modification of the quantitative biuret method developed for serum protein by Gornell.[22] Three ml. of the biuret reagent (0.15 per cent copper sulfate, 0.6 per cent Na-K-tartrate and 3 per cent NaOH) are added to an aliquot of the test mixture containing approximately 1 to 2 mg. of protein in 3 ml. of water. This is allowed to stand for 10 minutes and then read in the Klett-Summerson colorimeter against a blank containing no protein. This method was standardized against data obtained with the Kjeldahl method for determining protein nitrogen. It is accurate to within 10 per cent.

PROPERTIES OF CHOLINE ACETYLASE

A. STABILITY OF THE ENZYME

Choline acetylase isolated from the Squid ganglion is unstable at room temperature or above. It loses its activity at 55° C. in 10 minutes; when kept at 30° C. in the absence of its substrate acetyl CoA, it loses from 15 to 50 per cent of its activity in 30 minutes. The loss is much smaller, of course, when the enzyme is kept concentrated than when it is diluted, as it must be, during pre-incubation. As mentioned above, however, it loses only one-third of its activity when kept frozen at —20° C. for one-and-one-half months. Addition of EDTA and cysteine to the extract before it is stored does not prevent the loss in activity. Addition of these reagents during the course of the reaction greatly increases the activity of the enzyme extract.

B. pH AND TEMPERATURE

Choline acetylase has a pH optimum of 6.9. The activity falls off faster on the alkaline side than on the acid side. The enzyme preparation is quite unstable below pH 6.0, but seems to be somewhat more stable on the alkaline side (at least up to pH 8.2). However, acetyl CoA is rapidly split above

about pH 8.5, so that activity measurements beyond these limits are not feasible. The temperature optimum for activity is at 30 to 32° C.

EDTA was present in these determinations. Liberated CoA was assayed by the nitroprusside method.

C. EQUILIBRIUM

The equilibrium of the reaction seems to be completely in the direction of the formation of acetylcholine. Assuming Stadtman's value of 0.85 μM of CoA per mg of Pabst CoA as discussed above, and a 100 per cent yield of the acetylated SH as measured by the hydroxamic acid method, there is a stoichiometric conversion of acetyl CoA to acetylcholine. This might have been expected on the basis of the very high energy content of the thiolester and the much lower energy content of the alcohol ester (acetylcholine). Very high enzyme concentrations were used, and relatively low acetyl CoA concentrations.

D. RATE OF REACTION

The activity of the enzyme was found to be fairly linear with enzyme concentration up to 440 μg protein per ml at 30 minutes incubation time and up to 220 μg at 120 minutes. However, it is not linear with time even at short time intervals. This deviation from linearity increases with higher initial activity.

The K_m for choline is 5×10^{-4} M. Since a concentration of 2×10^{-2} M was used per tube, and since less than 1 μM of acetylcholine was formed during the reaction, the choline concentration could not be the limiting factor for linearity. The K_m for acetyl CoA for the 11 minute values is 1.6×10^{-3} M. Here too the decrease in acetyl CoA concentration could not cause the sharp decrease in the rate observed, since the initial concentration was 3.5 μM per ml.

Another important fact in regard to the absence of linearity with time is that, in the presence of catalytic amounts of CoA, acetylphosphate and phosphotransacetylase, there is a marked increase in the rate of the reaction. In the transacetylase containing system, 0.2 μM of acetyl CoA is the highest concentration ever available, and yet the reaction is linear. A concentration of 0.2 μM per ml is considerably lower than the K_m at 11 minutes of 1.6 μM, or the acetyl CoA concentration available after 6 minutes, or even after 30 minutes, in systems using acetyl CoA directly as a substrate. This, therefore, again indicates that the initial acetyl CoA concentration of 3.5 μM per ml is not the limiting factor for maximum choline acetylase activity.

The possibility that acetylcholine or CoA act as inhibitors in the reaction was also investigated. Both inhibit, but at concentrations much higher than those produced during the course of the reaction. With acetylcholine, the inhibition is of the same order of magnitude as that of other inhibitors presumably competing with choline (Table 6). This will be more fully discussed below.

TABLE 2. ENZYME ACTIVITY IN THE PRESENCE OF CYSTEINE, CoA, OR ACETYL CoA, ADDED ALONE OR TOGETHER

Enzyme extract containing 160 μg of protein, was incubated for 20 minutes at 30° C. in a volume of .29 ml with the following components in μM/ml final concentration: K-phosphate buffer pH 7.0 100, EDTA pH 7.0 2, and, where indicated, cysteine 5, CoA 3.4, and acetyl CoA 3.4. The enzyme extract used was six weeks old. Then the following were added: TEPP .13 μM/ml, acetyl CoA (where not previously added) 3.4, and choline chloride 20, the final volume brought to .5 ml, and the test mixture incubated at 30° C. Acetylcholine formed was determined by biological assay. Values in μM/ml.

Additions	*ACh formed in 6 mins.*	*Per cent change*
None	.2	....
Cysteine	.38	+90
Cysteine plus CoA*	.26	+30
Cysteine plus AcCoA*	.25	+25

* CoA was added after cysteine had been pre-incubated with the enzyme; AcCoA was pre-incubated together with the cysteine and enzyme extract.

CoA at a concentration of 0.9 μM per ml stimulated the enzyme activity; at a somewhat higher concentration (4.4 μM) it was lightly inhibitory. However, it was found that cysteine and other SH reducing agents greatly activated the enzyme, even in the presence of EDTA. This cysteine activated enzyme preparation was inhibited 32 per cent by 3.4 uM of added CoA (Table 2). The extent of the activation and inhibition by CoA varies with different enzyme preparations, and with the length of time that the enzyme extract is stored. CoA activates an old extract much more than a fresh one. The initial accelerating effect of CoA may therefore have been due to the reducing power of its sulfhydryl group on the enzyme.

The inhibition of over one-third of the enzyme activity by CoA in the presence of cysteine and EDTA is consistent with Tabor *et al.'s*[97] figures for CoA inhibition of the amine acetylating enzyme. In that system CoA added in twice the concentration of the substrate, acetyl CoA, gave an inhibition of a little over one-third, while CoA added in 10 times the acetyl CoA concen-

tration inhibited the reaction almost two-thirds in the presence of sodium thioglycolate and EDTA. The substrate and inhibitor concentrations used in the choline acetylating system are, however, much higher than those used for the amine acetylase.

The apparent increase with time of the K_m of acetyl CoA may also suggest the accumulation of a competitive inhibitor. Some important information was obtained from experiments in which the phosphotransacetylase system (purified *E. coli* extracts, acetylphosphate, and magnesium chloride) was added to the acetyl CoA-choline acetylase system, and the acetylcholine formed assayed by bioassay (Fig. 1). No free CoA was added, but the CoA liberated during the course of the reaction was immediately reactylated. The results demonstrate that the added reacetylating system not only sharply increases the rate of acetylcholine formation, but also causes this rate to become linear.

The systems with and without the added phosphotransacetylase were also compared with and without added cysteine. This addition further speeds up the rate of the reaction in the presence of the transacetylase. In its absence, there is an initial increase in rate with cysteine, but this levels off and the enzyme activity decreases with time.

The increase in acetyl CoA concentration in the presence of the phosphotransacetylase system is not great enough to account for the sharp increase in activity and linearity of the choline acetylase system, especially during the first period of the reaction. The increased rate may therefore be due to the removal of the free CoA which is formed during the course of the reaction and which may act as an inhibitor. If an inhibitor of the acetyl CoA site were to accumulate during the course of the reaction, the apparent concentration of substrate necessary for half maximum enzyme activity would increase.

The maximum specific activity of the enzyme preparation obtained by adding the phosphotransacetylase system to the choline acetylase-acetyl CoA system, in the presence of cysteine, and assayed by bioassay agrees very well with the specific activity of the same enzyme preparation determined with catalytic amounts of CoA, no added acetyl CoA, and phosphotransacetylase, and assayed by the alkaline hydroxamic acid method.

The sharp decrease in the linearity of the isolated choline acetylase reaction with time in contrast to the strong increase in, and linearity of, enzyme activity in the presence of the phosphotransacetylase system raises an interesting problem. Free CoA inhibits the reaction of the enzyme with acetyl CoA in concentrations markedly higher (six to eight times) than those produced during the course of the reaction. A possible explanation is that CoA formed from acetyl CoA during the reaction may be better bound to

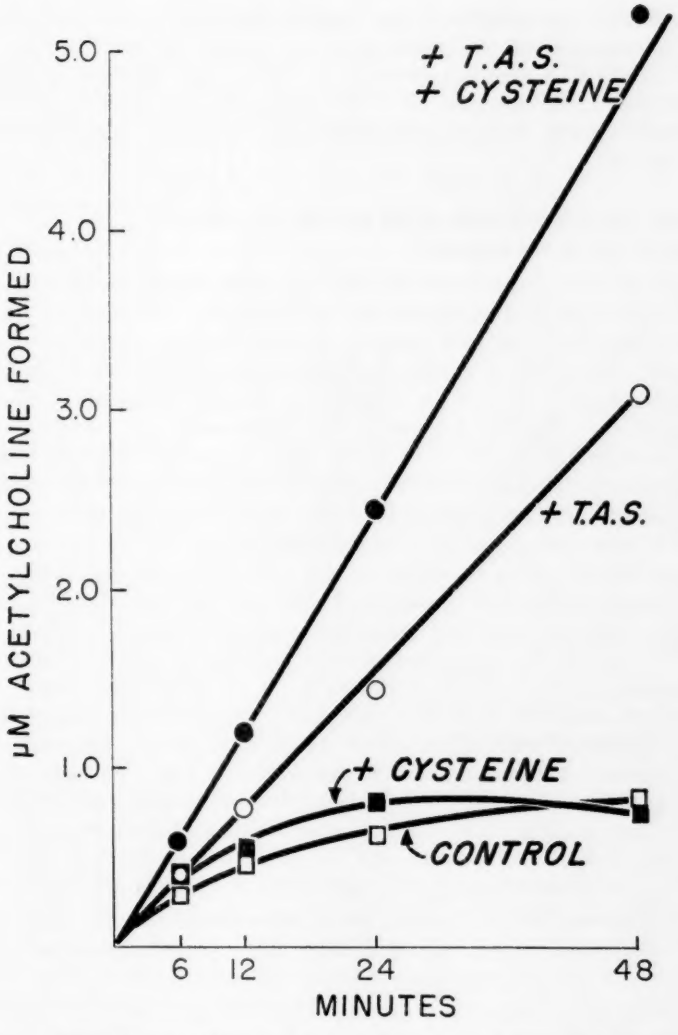


FIG. 1. Rates of acetylcholine synthesis in presence and absence of phosphotransacetylase. The control reaction mixture contains in addition to choline acetylase and choline only acetyl CoA. The transacetylase system (T.A.S.) contains instead of acetyl CoA acetylphosphate, CoA and Mg. The rates of the two systems are reproduced with and without addition of cysteine.

the enzyme than added CoA. If a system is added which pulls the CoA off, as for example the phosphotransacetylase system (which might have an affinity for CoA even though the coenzyme may still be partly associated with the choline acetylase) the reaction continues linearly. This latter test system might be close to the conditions under which the enzyme functions in the cell.

E. THE SULFHYDRYL NATURE OF THE ENZYME, AND POSSIBLE MECHANISMS OF THE REACTION

It had been suggested in 1943 that the crude choline acetylase system contained sulfhydryl groups essential for its activity.[87] However, the results obtained at that time were based on rat or guinea pig preparations which contained free CoA. When the coenzyme was found to contain a free functional sulfhydryl group,[45] the existence of essential SH groups in choline acetylase itself could be questioned. The answer was readily found by starting directly with S-acetyl CoA as the substrate, instead of synthesizing it from free CoA and an acetylating system during the course of the reaction.

Known sulfhydryl inhibitors were then used to investigate whether or not the enzyme contained an essential sulfhydryl group.[8] Choline acetylase was unequivocally shown to require a free SH for functioning (Table 3). Therefore, activation of the enzyme by removing traces of metals in solution which might react with SH groups was attempted. Addition of 2 μM per ml of EDTA to the test mixture gave a 37 per cent increase in acetylcholine formation in the presence of phosphate buffer. A 450 per cent increase was observed when two times recrystallized tris buffer (tris [hydroxymethyl] aminomethane) was used instead of the phosphate buffer. This suggested that a metal contaminant had been present in this buffer. As both the enzyme and the free coenzyme have been shown to contain functional sulfhydryl groups, added EDTA should presumably have two effects on the test system: it should not only keep the SH on the enzyme intact, but the SH on the CoA liberated during the course of the reaction should remain free.

As functional SH groups may have been oxidized during the purification procedure, the enzyme was pre-incubated with cysteine. The activity was markedly increased beyond that observed with EDTA. The cysteine effect, however, might have been a specific one rather than a general reducing action. The SH group may have removed another group blocking an enzyme sulfhydryl. Therefore, choline acetylase was treated with sodium borohydride which readily reduces disulfide bonds, and being a small molecule, might be expected to easily penetrate proteins. It had no effect at all on the enzyme, neither stimulating nor inhibiting it.

TABLE 3. EFFECT OF SH INHIBITION ON CHOLINE ACETYLASE

The test mixture contained, in μM per ml, the following components: choline chloride 20, acetyl-CoA 3.5, K-phosphate buffer 100, pH 7, TEPP .13. Inhibitor as indicated. One-hundredth ml. enzyme, containing 150 ug protein, was added to the test mixture which had .5 ml as final volume. When iodoacetate was the inhibitor, the test mixture contained in addition 4 μM per ml versene; in case of p-chloromercuribenzoate and iodosobenzoate 2 μM/ml; no versene in case of $CuSO_4$. The acetylcholine formed was determined by biological assay. The activity of the control was about 80 μg in 10 minutes and about 150 μg in 30 minutes.

Compound	*M conc.*	*Inhibition per cent*	
		10′ incub.	*30′ incub.*
Iodoacetate	5×10^{-5}	0	0
	5×10^{-4}	35	27
	5×10^{-3}	60	64
	5×10^{-4}	36	20
	1.5×10^{-3}	63	48
	4.5×10^{-3}	78	64
p-Chloromercuribenzoate	4×10^{-5}	96	94
	2×10^{-4}	100	100
	1×10^{-3}	100	100
	4×10^{-7}	12	6
	2×10^{-6}	40	18
	1×10^{-5}	69	48
Iodosobenzoate	1×10^{-5}	48	34
	1×10^{-4}	63	50
	1×10^{-3}	70	65
	1×10^{-6}	16	12
	1×10^{-5}	50	39
	1×10^{-4}	69	62
$CuSO_4$	1×10^{-6}	13	10
	1×10^{-5}	80	83
	1×10^{-4}	100	100
	2.5×10^{-6}	38	30
	5×10^{-6}	60	59
	1×10^{-5}	84	86

Glutathione stimulates the enzyme as well as does cysteine (Table 4), pantetheine perhaps even better (it was used in lower concentration); pantethine strongly inhibits it, and sodium formaldehyde sulfoxylate ($CH_2OHOSONa$), which has a reducing sulfur that is not an SH, activates choline acetylase to a slightly lesser extent than does cysteine. The

TABLE 4. EFFECT OF SH AND OTHER REDUCING AGENTS

Enzyme extract, containing 160 μg of protein, was pre-incubated for 20 minutes at 30° C. in a volume of .11 ml with the following components in μM/ml final concentration: K-phosphate buffer pH 7.0 100, EDTA pH 7.0 2, and, where indicated on Table, reducing agents (except pantetheine) 5, pantetheine 2.3, pantethine 5. Then, TEPP .13 μM/ml, choline chloride 20, and acetyl CoA 3.4 were added, the final volume brought to .5 ml. and incubated at 30° C. Acetylcholine formed was determined by biological assay.

Reagents pre-incubated with enzyme	*ACh formed in 6 min. μM/ml*	*Per cent change Increase = + Decrease = −*
1. None	.36	....
Cysteine	.5	+41
Glutathione	.53	+50
Sodium formaldehyde sulfoxalate	.47 (corrected)	+32
Sodium borohydride	.34	−4 (0)
Sodium borohydride plus cysteine	.51	+44
2. None	.49	....
Cysteine	.60	+24
Pantetheine	.58	+18
Pantetheine plus cysteine	.7	+42
Pantethine	.13	−74
3. None	.33	....
Cysteine	.48	+45
Pantetheine	.47	+40

slight increase in the degree of activation when pantetheine and cysteine were added together may be due only to a higher concentration of SH groups.

Reactivation of o-iodosobenzoate inhibition was attempted with both borohydride and cysteine. Under the conditions used, reactivation was obtained with both compounds. It was quite weak with $NaBH_4$, whereas cysteine reactivation was stronger. The two reactivators are not quite comparable, however, as cysteine reduces the sulfhydryl groups on the enzyme

and also reacts with the ortho-iodosobenzoic acid, while $NaBH_4$ only reduces the sulfhydryl groups on the enzyme. Since cysteine also activates the system independently of its reactivation effect (Table 2), a quantitative evaluation of its reversal of o-iodosobenzoate inhibition is not possible.

The finding that choline acetylase is inhibited by low concentrations of compounds reacting with sulhydryl groups is of great significance in approaching the problem of the mode of action of the enzyme. The fact that incubation is necessary for maximum inhibition indicates a relatively slow interaction. It is of interest that Tabor *et al.*[97] have shown that the enzyme catalyzing N-acetylation of amines also contains an essential SH group. The acetyl-carrying enzyme, thiolase, also has sulfhydryl groups essential for its activity.[46, 93]

Most of the substances which activate choline acetylase are themselves sulfhydryl compounds. The one exception is sodium formaldehyde sulfoxylate. Pantetheine, which resembles CoA most, seems to be a somewhat more effective activator than cysteine. Since EDTA was present in all the test mixtures, the activation of the enzyme by these reducing compounds indicates that not only may the removal of a metal inhibitor be involved in the activation but also the reduction of the oxidized enzyme. However, the addition of sodium borohydride, which is a small and therefore presumably an easily penetrating molecule known to reduce sulfhydryl groups readily, does not increase the enzyme activity, although it does partially reactivate enzyme oxidized by ortho-iodosobenzoic acid. The activation by cysteine and EDTA appears only when these substances are present in the reaction mixture, but not when they are added during the preparation of the extract. Activation of the enzyme may therefore be due to the removal of an inhibiting group during the course of the reaction.

Pre-incubation of the enzyme with acetyl CoA results in increased enzyme activity when compared with controls kept at the same temperature for the same period of time. Usually, this increase is about 50 per cent in the first 10 minutes, but it varies very widely (from 5 to 170 per cent). Keeping the enzyme at 30° for 30 minutes with acetyl CoA also results in a slightly better activity (about 20 per cent) than that obtained with enzyme alone kept at a low temperature. When, however, the enzyme is preincubated with acetyl CoA and cysteine together, its activity drops to almost the level of the control. This may be due to the blocking of the cysteine action at the site of activity by the acetyl CoA and vice versa. However, significant amounts of acetyl CoA may break down during the course of the reaction under the conditions used.[79, 81]

Of other thiolesters tested, only acetyl pantetheine forms a very small amount of acetylcholine, about one-thirtieth as much as that obtained with

acetyl CoA at about the same concentration. Higher concentrations were not tested. Added pantothenate, ATP, and ADP did not affect the reaction rates.

Considering how essential the sulfhydryl groups on the enzyme are for its activity, and in view of the acetylthiolester structure of the "active acetate" intermediate, it is possible to suppose that a similar structure for the acyl-enzyme might be operative. An actual transfer of the acetyl group from the coenzyme to an SH group in the enzyme may be involved, releasing free CoA and free acetate. This would presumably take place even in the absence of choline.

Racker and Krimsky postulated such an acyl-S-enzyme intermediate in their scheme for phosphoglyceraldehyde oxidation,[96, 97] based on the requirement of the enzyme for glutathione as a prosthetic group and on the acetylation of the enzyme, prepared in gram quantities, by acetylphosphate as measured with the hydroxamic acid test; this intermediate was also assumed as part of the mechanism of thiolase action by Lynen *et al.* and Stern *et al.*[44, 45, 98] Tabor *et al.*[97] suggested that amine acetylation may also occur through a free acyl-S-enzyme intermediate. However, as Racker and Krimsky pointed out, free acetyl CoA is not involved in glyceraldehyde-3-phosphate dehydrogenase activity, and their suggested acyl-enzyme thiolester is a fixed system.

An acylated enzyme as an intermediate form was first postulated by Wilson for acetylcholinesterase,[99] although the point of attachment here cannot be an SH group.

An alternative mechanism of the synthesis of acetylcholine could be formulated. It would not postulate a *free* acyl enzyme intermediate, but it would involve the enzyme sulfhydryl group, as follows:

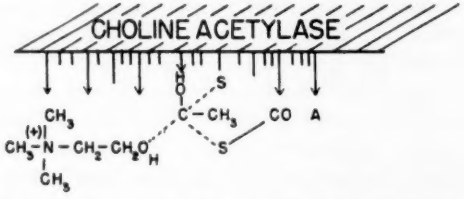


FIG. 2. Schematic presentation of the interaction between the protein of choline acetylase, choline, acetate, and CoA.

The coenzyme and the enzyme may have several interacting groups which, acting together, may fix the acyl group into a very specific steric position. Then the carbonyl carbon could become associated with both the enzyme

and CoA sulfhydryl groups at the same time. The choline, which is attracted to a nearby site, would then form a compound by bonding its oxygen with the carbonyl carbon, thus simultaneously releasing the CoA. Inhibitors could interfere with the reaction by blocking any or all points of interaction of acetyl CoA and enzyme.

TABLE 5. FORMATION OF BUTYRYLCHOLINE AND THE INHIBITORY EFFECT OF BUTYRYL CoA ON ACETYLCHOLINE SYNTHESIS

Enzyme extract (.01 ml) containing 150 μg protein was pre-incubated for 20 minutes at 30° C. in a volume of .08 ml with the following components in μM/ml final concentration: K-phosphate buffer pH 7.0 100, EDTA pH 7.0 2, and cysteine 1. Then .13 μM/ml of TEPP, acetyl CoA, and butyryl CoA in the concentrations indicated below and choline chloride 20 μM/ml were added, the final volume brought to .5 ml, and incubated at 30° C. The two choline esters formed were determined by biological assay.

			μM formed in 10 min. of			
Exp. no.	*μM AcCoA added*	*μM BuCoA added*	*ACh*	*BuCh*	*Together in equiv. of Ach*	*Per cent inhibition of Ach (apparent)*
1	3.1	..	.31	..	..	..
	3.1	3.1	..	..	.18	42 (uncorrected)
						corr.f. BuCh form.
2	1.7	..	.35	..	..	..
	..	1.6	..	.07	..	..
	3.4	..	.47	..	..	..
	..	3.2	..	.08	..	..
	1.7	1.6	..	..	.33	
	1.7	3.2	..	..	.26	
	3.4	1.6	..	..	.41	
	3.4	3.2	..	..	.36	

As noted above, acetyl pantetheine is a far weaker acyl donor than the whole acetyl CoA molecule, while acyl thiolesters not related to CoA in any way do not serve as donors at all. One factor for this stronger interaction between CoA and enzyme, compared to pantetheine, may be its stronger binding to the enzyme.

F. SPECIFICITY

In earlier experiments[7] the specificity of the enzyme for the amino alcohol in regard to its degrees of N-methylation was found to be choline >> (16 x) dimethylethanolamine > (3 to 4 x) monomethylethanolamine. In

regard to the acid moiety, only the acetyl and propionyl group formed the corresponding ester. Butyrylcholine formation was too low to measure. Benzoyl CoA was also inactive.

With improved techniques, synthesis of butyrylcholine, but not of benzoylcholine, was demonstrated. Butyrylcholine contracts the frog's rectus 80 per cent as strongly as acetylcholine, and this method was used to confirm butyrylcholine formation (see Table 5). Tested with the nitroprusside method, butyrylcholine synthesis was about 10 to 20 per cent as high as acetylcholine synthesis.

The specificity of the acetylase and esterase in regard to chain length is similar. The acetyl CoA is only a slightly more active acylating agent than the propionyl thiolester, but is much better than the butyryl CoA in synthesizing the respective esters.

Although the reaction of the enzyme with the substrates is not completely specific, it is quite marked enough so that the function of the enzyme in the cell appears to be essentially acetylcholine and perhaps propionyl choline synthesis. Of the other substrates tested and found to react to even a small extent, apparently only butyryl CoA seems to acylate choline *in vivo*, as only butyrylcholine has been detected in brain extracts.[39] The availability of butyryl CoA to the choline acetylase, however, may be small, but since this ester is a poor substrate for acetylcholinesters, it is possible that detectable amounts are built up in time even though it is synthesized slowly by the acetylase. Succinyl CoA, which is involved in carbohydrate oxidation as well as in other systems, does not form detectable amounts of the choline ester.

G. INHIBITION RELATED TO THE NATURAL SUBSTRATES

Several of the inhibitors of choline acetylase activity in the isolated enzyme reaction have already been discussed above. The action of choline acetylase may be further elucidated by studies of inhibitors reacting specifically at each of the presumed sites of substrate-enzyme binding. Compounds having structures similar to the natural substrate were chosen, since these might have an affinity for the enzyme even if they do not react. In this way, enzyme specificity in relation to binding power, as opposed to reactivity, may be studied separately. This has been done for the acetylcholinesterase by Wilson.[101]

Because the reaction utilizing acetyl CoA as a substrate was not linear, experiments designed to indicate whether added inhibitors acted competitively or noncompetitively could not yield definitive results.

1. *Inhibitors competing with choline*

a. *Simple methyl amines.* As shown in Table 6, tetramethyl ammonium chloride inhibits at a concentration of 40 μM per ml more strongly than trimethylamine hydrochloride does at 80 μM per ml; dimethylethanolamine apparently inhibits even less (20 per cent) at 80 μM per ml. (However, as choline acetylase catalyzes the acetylation of high concentrations of this

TABLE 6. INHIBITORS PRESUMABLY COMPETING WITH CHOLINE.
A. METHYLATED NITROGEN COMPOUNDS

The test mixture contained the following components in μM/ml: choline chloride 5, K-phosphate buffer, pH 7.0 100, AcCoA 2.2 and 4.5, TEPP* (in acetylcholine inhibition) .035. Inhibitors as indicated. Two-hundredths ml enzyme, containing 260-290 μg protein was added per ml test mixture. Incubation temperature was 31° C. Liberated SH was assayed by the nitroprusside method. Dimethylamine did not inhibit at 80 μM/ml.

Compound	*Concentration* μM/ml	*Per cent inhibition* 15'	30'
Tetramethylammonium Cl	30	63	51
	40	42	32
	40	54	53
	20	28	23
Trimethylamine HCl	80	33	26
	40	13	4
Dimethylethanolamine	80	21	23
Acetylcholine Cl	50	40	42

* It was necessary to add TEPP to the reaction mixture when acetylcholine was studied to inhibit esterases which are still present in the enzyme preparation used.

latter alcohol, the observed 20 per cent inhibition of the liberation of free CoA does not indicate its total inhibition of acetylcholine synthesis.) Dimethylamine hydrochloride does not inhibit at all at the latter concentration. Pre-incubation has no effect on the inhibition. Thus, the decrease of inhibitory strength with a decreasing number of methyl groups attached to the nitrogen is fairly consistent with the behavior of acetylcholinesterase and is of approximately the same order of magnitude. In both cases the methyl groups apparently increase the attraction by Van der Waal's forces.

As tetramethyl ammonium chloride was two to three times better as an inhibitor at pH 7.0 than trimethylamine hydrochloride (pK = 9.87), a change in protein configuration due to the extra methyl group may be a

factor in the interaction of choline acetylase with the inhibitor. Although this had been considered improbable for the esterase,[101] more recent data show that the tetramethyl compound is also a significantly better inhibitor of this enzyme. The absence of any inhibition by 80 μM per ml of dimethylamine hydrochloride (pK = 10.72) is further support for the assumption of Van der Waal's forces being a factor in the attraction of methyl groups.

TABLE 7. INHIBITORS PRESUMABLY COMPETING WITH CHOLINE.
B. PROSTIGMINE AND ITS TERTIARY ANALOGUE (T-BASE)

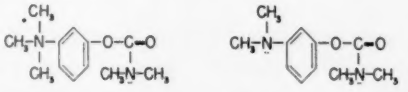
The test mixture contained the following components in μM/ml: Choline chloride 5, K-phosphate buffer, pH 7.0 100, acetyl CoA 2.2 in 2d T-Base experiment, 3.1-3.4 in others. 0.01 ml enzyme, containing 130-150 μg protein was added to the test mixture which had a final volume of 0.5 ml and was incubated at 21° C. Liberated SH was assayed by the nitroprusside method. The control had an activity of .6 μM SH liberated in 15 minutes, and 1.05 μM in 30 minutes with pre-incubation. .72 and 1.25 μM respectively without pre-incubation. The addition of EDTA did not significantly affect these results.

Compound	*Concentration μM/ml*	*Pre-incubation minutes*	*Per cent inhibition for incubation period of: with pre-incub. 15′*	*with pre-incub. 30′*	*without pre-incub. 15′*	*without pre-incub. 30′*
Prostigmine -Bromide	80	..	..	..	54	50
	20	..	..	..	15	24
	40	30	20	34	24	38
T-Base	5	10	33	44	17	12
		15	69	64	28	14
		30	67	73	17	12

b. *Prostigmine and its tertiary analogue.* The inhibitory power of the aromatic compound prostigmine (80 μM per ml gives 50 per cent inhibition) is lower than that of the simple tetramethylammonium ion. This is strikingly different from the finding with the esterase. In the latter case, prostigmine inhibits at 10^{-7} M or at 100,000 times lower concentration. This very strong inhibition is not surprising in the case of the cholinesterase.[108] Prostigmine is bound to the acetylcholinesterase at both the anionic and esteratic sites, as the distance between its quaternary nitrogen and its carbonyl-C is about the same as in acetylcholine.

The much greater inhibitory power of the analogous tertiary amine as opposed to the quaternary prostigmine (10 to 20 times) (Table 7) is also different from the situation with the esterase. For that enzyme, prostigmine has a K_I of 1.6×10^{-7}, while the tertiary analogue is about one-hundredth as strong.[104] Conversely, the tertiary compound requires only 5 μM to inhibit the choline acetylase reaction 70 per cent, but this strong inhibition takes place only after the inhibitor is pre-incubated with the enzyme for 15 to 30 minutes. This increase of inhibitory strength on pre-incubation suggests that another interaction, besides simple electrostatic attraction to a negatively charged area on the enzyme, must be involved.

This was confirmed when it was found that if acetyl CoA were pre-incubated together with the enzyme and this tertiary analogue, the inhibition was reduced to less than half of what it was when the enzyme was treated with the tertiary compound alone. Choline, on the other hand, did not protect the enzyme at all against this inhibitor. The protection against this inhibition by acyl CoA, together with the fact that contrary to most presumed choline site inhibitors the inhibition by the tertiary analogue of prostigmine increases with pre-incubation, suggests that it may have a two-headed inhibitory action on the acetylase. It probably inhibits at the choline site, somewhat as trimethylamine does. But the greater part of the inhibitory reaction seems to involve the acetyl-CoA site. The three major characteristics of its inhibition, (a) low inhibitor concentration, (b) increase in inhibitory power with pre-incubation, and (c) decrease in inhibition in the presence of acetyl CoA, all point in this direction. In the reaction with the acetyl CoA site a more specific complex formation may occur between inhibitor and enzyme. The free electron pair on the nitrogen, due to the absence of the extra methyl group, could affect the electron distribution of the carbonyl group:



c. *Acetylcholine.* The acetylcholine inhibition shown (Table 6) is of the same order of magnitude as that shown by tetramethylammonium chloride and is probably due to competition with choline, since both contain a quaternary nitrogen. The much smaller concentration of acetylcholine formed during the course of the reaction is certainly in itself not enough to cause a decrease in enzyme activity. It might be, however, that this inhibition, plus that due to CoA, are more effective together than separately.

No mass action effect of acetylcholine accumulation is to be expected, as the $-\Delta F^{\circ}$ is close to 10,000 calories, and the equilibrium is all the way toward ester formation.

d. *D-tubocurarine and other diquaternary compounds.* If, as in acetylcholinesterase, there were more than one negatively charged functional centers in close proximity to each other, diquaternary compounds would be expected to inhibit as effectively as the monoquaternary compounds with the square root of the concentrations needed for the latter. D-tubocurarine actually is quite potent but, as with the tertiary analogue of prostigmine, only after pre-incubation. Whereas 5 uM per ml inhibit only 5 per cent

TABLE 8. INHIBITORS PRESUMABLY COMPETING WITH ACETYL CoA

The test mixture contained the following components in μM/ml: Choline chloride 20, acetyl CoA 1.6, K-phosphate buffer pH 7.0 100. Inhibitors as indicated. One-hundredth cc. of enzyme containing 120 μg protein was added to the test mixture which had a final volume of 0.5 ml. The enzyme and inhibitor were pre-incubated at 20° C., and the final volume was incubated at 30° C. Liberated SH was assayed by the nitroprusside method. The control for 18 minutes had an activity of .39 μM of SH liberated.

Compound	*Concentration μM/ml*	*Incubation period min.*	*Per cent inhibition pre-incubated*	*Without pre-incubation*
Benzoyl CoA	.42	18	35	26
		36	23	13.5
		30	26	17.5
		60	20	13.9
Na hippurate	70.	18	25	0
		36	24	0

without pretreatment, the inhibition goes up to 60 per cent after 15 minutes of pre-incubation. However, there is a marked increase in acidity during the course of the reaction, even in high buffer concentrations, which probably accounts for some of this inhibition.

On the whole, there seems to be no increase of inhibitory strength with diquaternary compounds, in contrast to the results obtained with the esterase.[5] Decamethonium bromide inhibits 25 per cent at a concentration of 25 μM per ml, and succinylcholine 20 per cent at 40 μM per ml. Thus, there is no indication of the existence of a second negative group near the active site.

Inhibitors of choline acetylase at the choline site generally act in relatively high concentration, and the length of time that the enzyme and inhibitor are pre-incubated together has no effect. The major attracting forces are presumably Van der Waal's forces, and possibly also electrostatic interactions.

2. *Inhibitors competing with the acyl group*

a. *Butyryl CoA.* The analysis of butyryl CoA inhibition is complicated by the formation of butyryl choline. Taking this reaction into account, the per cent of inhibition ranges from 15 to 40 per cent, although an exact quantitative evaluation would be difficult (Table 5). The inhibition varies with butyryl CoA concentration, but an analysis of the results to determine whether or not the inhibition was competitive was inconclusive. On the basis of the inhibition of acetylcholine formation in the presence of butyryl CoA, acetyl CoA seems to be bound to the enzyme about twice as well as butyryl CoA when equal concentrations of the two acyl CoA's were used.

b. *Benzoyl CoA.* With benzoyl CoA, no formation of benzoylcholine could be detected, at least at the low concentration at which this acyl CoA was added. But inhibition of acetylcholine formation was definitely established (Table 8).

Sodium hippurate, at a much higher concentration than benzoyl CoA (about 150 times as high) also inhibits about 25 per cent, but only if it is pre-incubated with the enzyme. Succinyl CoA is quite unstable and breaks down rapidly during the course of the reaction. It neither inhibits acetylcholine synthesis nor is it utilized for succinylcholine formation.

The inhibition of acetylcholine formation at the acetyl CoA site has markedly different characteristics from that at the choline site. Butyryl and benzoyl CoA inhibition takes place at a concentration of about one order of magnitude lower than the concentration of inhibitors of the choline site. Another difference between the two is the increase of the inhibitory action on the acyl-CoA center, but not on the choline center, by pre-incubation of the reagent with the enzyme. This is clearly evident in the case of the action of benzoyl CoA, but hippuric acid and sulfhydryl inhibitors could also belong to the compounds acting on the acyl-CoA site. The incubation may favor complex formation or an interaction between the coenzyme and the choline acetylase at several points.

H. EFFECT OF IONS

The effect of ions, except in the case of metals which react with the sulfhydryl group, is negligible. At a concentration of 10 μM per ml, calcium ions inhibit the reaction about 10 per cent, manganese 20 to 30 per cent.

The presence or absence of sodium or potassium ions, at constant ionic strength, has no effect on the reaction.

BIOLOGICAL SIGNIFICANCE OF THE SUBSTRATE SPECIFICITY OF CHOLINE ACETYLASE

The sharp specificity of the enzyme for the quaternary trimethyl aminoethanol (choline) when compared with the analogous di- and monomethyl alcohols indicates that the third methyl group of choline is very important for enzyme activity. This is of great interest. It may be assumed that the methyl groups of the nitrogen contribute to the attraction of the substrate by Van der Waal's forces. This has been demonstrated and quantitatively evaluated by Wilson for the acetylcholinesterase.[7, 101, 108] In this latter enzyme, the third methyl (fourth alkyl) group does not contribute as much to the binding as the other methyls, whereas the hydrolytic activity of the esterase is markedly higher with acetylcholine (more than twice) than with its tertiary analogue. In the case of choline acetylase, the presence of the third methyl group has an even more pronounced effect, the rate of acetylation being increased about 16 times. Since the quaternary nitrogen group has a spherical shape, it may be that for full activity the enzyme protein has to envelop the molecule, and that this change of configuration increases the enzyme efficiency.

At least one of these proteins (acetylcholinesterase)[80] has been found to be localized in the neuronal sheath. It is probable that the other proteins with which acetylcholine interacts are also in the nerve membrane. Therefore, a change in the structure of a protein reacting with acetylcholine could cause a change in the arrangement of charges on the surface of this protein, and therefore a redistribution of charges in the membrane. This might allow sodium ions, which were previously repelled, to penetrate through the membrane.

The possibility that the effectiveness of the quaternary ion is due to its action on the configuration of the protein to which it is bound is supported by recent work on the receptor protein. A series of compounds related in structure to acetylcholine, which do not inhibit esterase activity but which do block conduction, were found. Of these, the majority of the tertiary compounds did not depolarize the membrane; in contrast, most of the quaternary compounds tested depolarized it. The former group thus acted as receptor inhibitors, the latter as receptor activators. These effects on the receptor are consistent with the results which one would expect from the studies of the two enzymes of the system in solution.

ACKNOWLEDGMENT

The author wishes to express her sincerest gratitude to Professor David Nachmansohn for his encouragement, interest, and advice throughout the course of this work. She would also like to thank Professor Irwin B. Wilson for many helpful discussions and suggestions.

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The Board of Editors

ERNEST POLLARD* | *Biophysics Department, Yale University*

INACTIVATION OF VIRUSES FOR VACCINE PURPOSES USING IONIZING RADIATION AND HEAT**

Several attempts have been made to use ionizing radiation as a means of inactivating viruses so that they can be employed as killed vaccines. Success has been achieved with a rabies virus, and trials have also been made with poliomyelitis virus.[6, 17] These methods have not been used for practical purposes, partly because of their novelty, because of the expense in the original setting up of the equipment, and partly because successful vaccines have been produced by other means.

In the light of modern studies of radiation action on viruses, it seems worth while to consider the relative advantages of ionizing radiation for inactivating viruses. We wish to see whether the expense and elaboration necessary might be practical. To do this we need to evaluate the fundamental studies of the action of ionizing radiation on viruses. This work has been in progress since 1937 and was begun separately in three nations by Holweck and co-workers in Paris, Lea and co-workers in England, and Gowen and Price in the United States.[15] The use of ionizing radiation to measure virus size, in particular, has been exploited by Bonet-Maury.[5]

A large share of the work in the Biophysics Department at Yale University for the past six years has been on the inactivation of viruses by ionizing radiation. This study has been concerned with the effects of radiation in many properties of bacterial, plant, and animal viruses. To summarize the results we can say that the infectivity property of viruses is highly sensitive to ionizing radiation. It can be characterized in terms of relative volume by saying that the sensitive volume for inactivation of a virus by ionizing radiation is rarely less than 1/20 of the whole virus. In terms of densely ionizing particles such as deuterons or alpha particles the sensitive area which is then concerned with such inactivation is very rarely less than one-half of the area of the whole virus.

In strong contrast to the infectivity is the sensitivity of serological combination. For tobacco mosaic virus sensitivity is very low, so that measurements have hardly been possible because of the side-effects produced by the

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** Assisted by the Office of Naval Research and the Atomic Energy Commission.
Received for publication April 23, 1956.

intense bombardment. Putting the relative sensitivities into numbers which are familiar to all, one can say that the molecular weight for inactivation of the infectivity of a virus corresponds to a molecular weight of about 30,000,000. By this we mean that an ionization within an object of molecular weight 30,000,000 would cause inactivation. On the other hand, the serologically sensitive volume seems to be of the order of a molecular weight of 10,000. This is the basis for the use of ionizing radiation to prepare a vaccine. The passage of a heavy charged particle through a virus will inactivate at the most two surface antigens, one at the entrance and one at the exit, whereas it will almost certainly cause the inactivation of virus infectivity.

One very important series of experiments is lacking. This would be aimed at testing whether a virus which retains a given fraction of its serological combining power has the power to produce antibodies in the same degree. A very incomplete set of trials on T-1 phage was made in the author's laboratory by Jane Setlow, W. P. McNulty, Ethel Tessman, and C. Woese.[9] It was shown that deuteron bombarded, wet heat inactivated, and dry heat inactivated T-1 which were not able to produce plaques nevertheless elicited good antibody response. The work of Traub, Friedemann, Brasch, and Huber on rabies virus indicated that for doses between 1.5 and 4.7 million rep the resulting vaccine had dropped somewhat in potency against challenge virus. The loss of the ability to elicit antibodies could perhaps be analyzed to correspond to a molecular unit of 45,000 molecular weight.[10] However, the experiments were not designed to test this particular point. Since it is by no means a certain conclusion that the sites on the molecule which combine with antiserum are the same as those which elicit production of antibodies, experiments should be set up specifically to test this point. Until then, we can take as a trial hypothesis that the fraction of a virus needed to elicit antibodies from an animal is the same as that which combines with antiserum.

Studies in the laboratory have also shown that there is strong combined thermal and radiation action.[1-4] This is most clear in the case of infectivity and has been demonstrated satisfactorily for one virus and for a number of enzymes.[11,18] The pattern of behavior of such enzyme molecules is quite clear, in that the combined effect of ionizing radiation and heat produces a more slowly varying thermal inactivation which starts at a lower temperature than ordinary thermal inactivation. Thus bombarding with ionizing radiation, at about 10° below the temperature at which heat alone will cause inactivation of a virus, will produce a considerably greater effect on the virus than similar bombardment at low temperatures. The effect of heat on the serologically combining portion of the virus has also been studied, and it

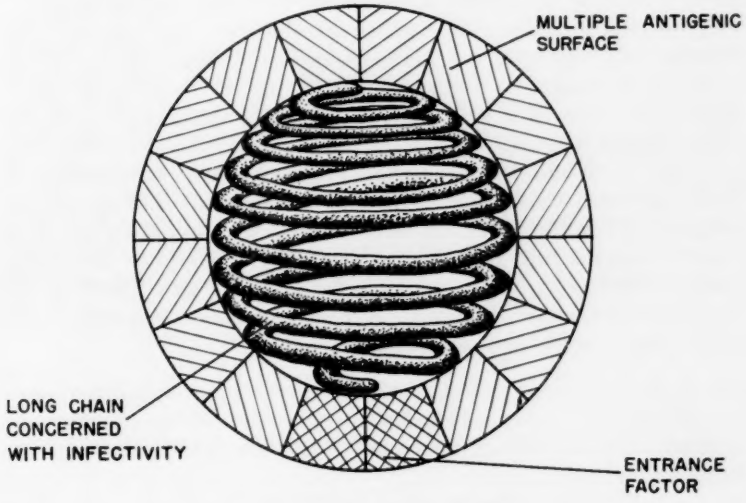
it clear that the thermal sensitivity of the virus surface is less than that of the infective center, but that serological sensitivity changes more rapidly with temperature than infectivity. Thus a virus which is inactivated at over 100° in the wet state could conceivably lose its serological properties before it loses its infectivity, though both will be inactivated very rapidly. On the other hand, a virus inactivated slowly at 50° is likely to lose its infectivity without any loss of serological properties. By combining the two it should be possible to irradiate a virus at a temperature which is insufficient to inactivate it thermally, but which in the presence of ionizing radiation will potentiate the action considerably, so that a strong combined effect on infectivity will result with very little effect on the serological activity.

Of great importance in the production of a killed vaccine is the inactivation of the whole population of the virus. Virus preparations such as polio, produced by ordinary methods, usually fail to show a simple logarithmic type of inactivation. This is illustrated in the thermal inactivation work of Kraft and Pollard[8] which shows that the polio virus has two components, one of which activated quite slowly and at rather high temperatures. It is necessary, however, to inactivate all components, including the insensitive, and if bombardment is used, this means we must bombard much harder than would at first seem necessary. The result of this is that the antigenic part of the virus is damaged. To some extent the use of heavily ionizing particles will give more simply logarithmic inactivations and so require a relatively smaller bombardment for the destruction of infectivity. The best method seems to be the combined use of heavily ionizing radiation and thermal inactivation. These two together seem to exert an action which reduces the less sensitive component markedly in amount and permits the damage of the infective part of the virus without damage to the surface of the virus. This double method of inactivation has not been tried very much in a practical way but the theoretical reasons which underlie the methods of inactivation would suggest that it is by far the best method. The theoretical reasons are now given.

The inactivation of a protein or nucleic acid molecule by the direct action of ionizing radiation seems to be caused by the removal of an electron from an atom in a long chain. The positive charge so produced can be neutralized by an electron from a neighboring atom but this produces a positive charge nearby which in turn is open to neutralization with the attendant formation of a nearby positive charge. Thus, until the electron removed in the ionization process can return and remove the separation of charge, there is a rapid migration along the covalent bonds in the molecule. This rapid migration of a deficiency in electronic content causes also a migration of an anomalous bond all over the molecule, since each covalent bond depends on electron

exchange between pairs of atoms and an electron removed is no longer one which can exchange.

A molecule can survive such a migrating weak spot if no bond is opened which can be followed by a permanent alteration. In the series of experimental studies of ionizing action made by the Yale group, a large fraction of enzymes and antigens require more than one ionization occurring simultaneously to remove the biological activity. Evidently, two or three weak



migrating bonds are more effective than one. This is perhaps related to the need for a permanent alteration in a new configuration before inactivation takes place. Studies by Alexander, Black, and Charlesby[3] indicate that cross linking of an isobutyl polymer can take place. Possibly this is the process referred to as alteration in a new configuration.

In the case of a virus we are dealing with a model possibly something like the figure (which is schematic only). The ionizing radiation, to remove infectivity, must damage some vital unit. There are many of these, but undoubtedly the most vulnerable are the large nucleic acid, or nucleoprotein, units in the virus center. The breaking of these long chains by radiation can be thought of as a mechanism which results in lost infectivity. Such breaking probably requires creation of three ion pairs situated closely together in the chain molecule.[15] Alternatively, the bonding between the long

chain coiled up molecule and the protein or lipid surface coat may become disrupted. If this happens, the integrity of the long chain may be broken. Thus heavy irradiation of the outer coat may produce effects on infectivity even though the inner chain is not hit. The vital damage, then, consists of either a triple ionization in the chain, or energy delivered to the coat which destroys the bonding to the coat and so weakens and breaks the chain.

It must be remembered that these remarks are in terms of a schematic model. Actually, such a model is not necessary for our conclusions; it is merely helpful in visualizing the sort of process which is taking place.

Turning now to the serologically active coating, it is clear that if it were highly multiple, the damage due to migrating weak bonds would be confined to one or two molecules in the surface. The net effect on the serological activity would therefore be very small. If we take two sets of figures for tobacco mosaic virus, data by Pollard and Dimond[18] indicate that the effective area of the infective part of the virus for deuteron bombardment is 2.1×10^{-11} cm^2 while the effective area for inactivation of the surface antigen is 1.5×10^{-14} cm^2.[14] By effective area, we mean the area within which a deuteron must pass in order to remove infectivity or the surface antigen. These figures mean that if n is the amount of virus infectivity left after bombardment by a dose of D deuterons per square centimeter, n_0 the original value before bombardment, then

$$\ln \frac{n}{n_0} = 2.1 \times 10^{-11} D.$$

Similarly, if A_0 and A are the serological affinities before and after bombardment, we have

$$\ln \frac{A}{A_0} = -1.5 \times 10^{-14} D.$$

If we now make the requirement that n/n_0 is 10^{-10}, to correspond to a safe vaccine, we have $D = 1.1 \times 10^{12}$ particles per square centimeter. The value of A/A_0 for such a dose is 0.97 or 97 per cent. Thus the antigenic surface is 97 per cent intact.

Such figures are very impressive but they leave out two very important practical features. The first is that most virus preparations are not homogeneous but contain populations of low sensitivity. Some of this low sensitivity may be related to the lack of synchrony in the original cells, but in any event it is a practical matter of great importance and is the major reason why ultraviolet light is difficult to use. The second factor is the presence in the preparation of quite dangerous compounds which can produce effects which are equal to or worse than the effects of the virus itself. The second feature

may militate against using such preparations for vaccines. The first merely makes it harder.

The practical problem, then, also contains the necessity for removing infectivity from *all,* including a resistant fraction, of the population. One way to do this is to bombard harder. This would certainly work for TMV. For polio, however, Pollard and Kraft[12] found a value of 6.0×10^{-12} cm^2 for the infectivity cross section and if we take the large antigen of southern bean mosaic virus or the serum blocking unit of T-1 bacteriophage to represent the (hitherto unknown) area of the poliomyelitis antigen cross section, we find that for reduction in infectivity by a factor of 10^{10} the antigenic surface is only 57 per cent intact. A greater degree of virus resistance thus necessitates the destruction of much of the antigenic surface. A second way of removing infectivity which is presented in this paper is to combine thermal and ionizing radiation inactivation. This gives a more favorable retention of the antigenic surface for a given destruction of the infectivity.

The thermal inactivation of a virus which has an homogeneous population follows in general first order kinetics, obeying the relation $\ln n/n_0 = k_1 t$, where k_1 is a reaction constant and t is the time in seconds. The reaction constant, in turn, can be described by the theory of absolute reaction rates as

$$k_1 = \frac{kT}{h} e^{\Delta S^{+}/R_e - \Delta H^{+}/RT} \ldots . (A)^{7}$$

where k is Boltzmann's constant, h is Planck's constant, T is absolute temperature, ΔS^{+} is the entropy of activation, ΔH^{+} is the heat of activation, and R is the gas constant. Values for ΔH^{+} for loss of infectivity are about 95,000, with values of ΔS^{+} of 207, while for loss of serological activity ΔH^{+} and ΔS^{+} are higher—more of the order of 165,000 and 370.

Where these two are well separated, thermal inactivation alone can produce a satisfactory vaccine. But again the multiple population problem introduces severe complication. Thus for MEF1 polio, Kraft and Pollard found at least two components in lyophilized virus, which inactivated in the dry state with ΔH^{+} of 28,800 and 20,800 respectively.

Returning for a moment to the theory of inactivation, it seems likely that something takes place in thermal inactivation which is similar to the mechanism suggested for ionizing radiation. That is, a breaking of bonds, with a permanent change of configuration, takes place. This has been specifically discussed by Augenstine.[8] Now for loss of infectivity the values of ΔH^{+} suggest a lower number of bonds broken than for serology. If we combine heat and ionizing radiation, the substance can be brought to a temperature at which it is very sensitive to radiation. Under such circumstances the cross section of a molecule which is at a temperature that would produce

thermal inactivation in about one hour is often much greater than the actual cross section of the molecule itself, indicating a transfer of energy into the molecule from nearby. By utilizing this principle, the infectivity cross section may be raised to a value in excess of the physical cross section of the virus, while the cross section for serological inactivation, having more sharply varying thermal constants, may not be changed at all.

If we take T-1 bacteriophage, for which some measurements exist, we have, for the dry state, for infectivity and serology the following figures.

$\Delta H+$ (infectivity) $= 27{,}500$ calories per mole; $\Delta S+$ (infectivity) $= 0$ calories per mole per degree

$\Delta H+$ (serology) $= 56{,}500$ calories per mole; $\Delta S+$ (serology) $= 57$ calories per mole per degree

At 105° C. the inactivation volume for ionizing radiation is 20×10^{-18} cm^3 (as compared with 3×10^{-18} cm^3 at 20° C.). Once again if we required that $n/n_0 = 10^{-10}$ we find, using the relation[16] for inactivation by fast cathode rays (or, essentially, x-rays also)

$$\ln n/n_0 = -VI$$

we find $$-10 \times 2.3 = -VI$$

or $$2.3 = 20 \times 10^{-18} I$$

$$I = 1.15 \times 10^{17} \text{ ionizing events per unit volume.}$$

Assuming an energy release of 110 e.v. is one ionizing event, we find a dose in rep of 1.9×10^5, a very reasonable figure. Such a dose can readily be given in 10 minutes by many quite modest sources of ionization. Heat alone will reduce the infectivity (according to equation (A)) with a reaction constant k_1 given by

$$k_1 = 2.07 \times 10^{10} \times 378\, e^{-27{,}500/1.98 \times 378}$$
$$= 7.8 \times 10^{-4}$$

The amount inactivated in 10 minutes is obtained from the relation $\ln n/n_0 = -k_1 \times 120$, and we find n/n_0 to be essentially unity. By substituting the numbers A/A_0 we obtain for serology a figure even closer to unity, meaning that no surface destruction at all has taken place. If wet inactivation is used, then at 60° C. the virus is sensitized to ionizing radiation to the same extent as in the above calculation. The thermal reaction constants are such that even less inactivation of any kind takes place in 10 minutes.

Thus we see, in one specific example for which actual data are available, that the effect of the double stress of ionization and heat is to inactivate the

sensitive infective part very thoroughly, while leaving the less sensitive parts essentially undamaged.

It therefore seems as though a trial of the inactivation of clean virus preparations by cathode rays, deuterons, or even intense x-rays, at a temperature gauged to be about that at which thermal inactivation to 37 per cent occurs in a few hours, should yield fully killed but actively antigenic virus preparations. With the modern availability of sources of ionizing radiation the cost should not be abnormally high, and the safety factors should be enough to render elaborate process control relatively unnecessary.

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YALE MEDICAL SOCIETY

November 12, 1956

VACCINATION AGAINST POLIOMYELITIS WITH ATTENUATED LIVE VIRUS. By Dorothy M. Horstmann, Joyce V. Deutsch, Joseph L. Melnick, and John R. Paul.

The purpose of this study is to procure a long-lasting immunity to polio and to develop an immune state of good protective quality. Heretofore, the Salk vaccine, a formalinized virus preparation, has been used; and it has been shown that this does not immunize the gastrointestinal tract itself although antibodies are produced. Sabin, using rodent-adapted virus, immunized orally. His subjects produced antibodies, but no illness occurred nor did any viremia.

The latter work suggested this study and the authors fed attenuated live virus to a group of individuals already possessing antibodies so that they could study the mechanism of reinfection. Type 3, Leon KP 34 poliovirus prepared by Sabin was used and large and small quantities were administered to subjects possessing either natural or Salk-induced antibodies. Throat swabs, stool specimens and blood samples were taken. Both neutralizing and complement-fixation type antibodies were present, and neither clinical disease nor viremia occurred. Virus was excreted in the stool in all cases, but positive throat cultures were obtained only when large doses of virus were given. Interestingly enough, excretion of virus lasted longer but at much lower levels in the Salk resistant individuals than it did in those possessing natural antibodies. No close correlation between the level of neutralizing antibodies and re-infection was found, however. Moreover, the excreted virus was still relatively avirulent with regard to humans, even though its virulence did increase somewhat. This fact, together with the lack of viremia, suggests a possible mode of differentiation of virulent from avirulent forms.

Tissue cultures grew some non-neurotropic types of virus, and it seems possible that a like form may be developed for a safer vaccine. In any case, the studies made thus far indicate that the gastrointestinal tract plays an important part in immunity and that the Salk vaccine plus oral administration of attenuated live virus leads to a more durable immune state.

J. A. O'NEILL

INSTRUMENTAL METHODS OF FETAL ELECTROCARDIOGRAPHY. By E. H. G. Hon.

Advances in medical care during the past two decades have resulted in a marked reduction of maternal mortality, whereas the reduction in stillbirths and neonatal mortality has been minimal. Many of the causes of perinatal death find a common basis in fetal anoxia, and if significant gains are to be made, a reliable means of determining fetal distress must be found.

The present study is concerned with the use of the fetal electrocardiogram for the recording of fetal heart rates throughout labor in order objectively to determine normal limits. In late labor there is often a marked slowing of the fetal heart rate with a prompt return to normal after a uterine

contraction: it is during this period that the first signs of fetal distress are often noted. This may be indicated by an abnormally low rate, or as an excessively long period before the fetal heart rate returns to normal. Abnormalities of fetal cardiac action may also be detectable in electrocardiographic configuration changes.

Another use of the present equipment is to detect fetal viability in cases of threatened abortion, questionable incomplete abortion, prematurity, and in cases where the obesity of the patient makes the use of the stethoscope unsatisfactory.

The current equipment allows the cancelling of the maternal complex which is present on the anterior abdominal wall together with the fetal electrocardiogram; and the recording of a separate fetal electrocardiogram makes it possible accurately to record the fetal heart rate by means of a decade counter.

The absence of clearly defined criteria for "fetal distress" point up the need for an extensive study of the fetal heart rate throughout labor, and during which there is correlation with the type of presentation, amount of cervical dilatation, force of uterine contraction, etc.

In an average labor, there are about 150,000 pulse intervals to be related to about 150 contractions. With current manual methods, it takes about 150 hours of technicians' time to process the data. To facilitate the study of this material, a semi-automatic data reduction system is being developed as part of the proposed comprehensive study.

It is hoped that this work will lead to a further understanding of fetal distress and hence to a reduction in perinatal mortality. JACK LEVIN

A RÔLE OF ERYTHROCYTES IN HOST DEFENSE. By Robert A. Nelson, Jr.

Continued investigations have been performed following the hypothesis that erythrocytes function as opsonizing agents *in vivo* and therefore may be considered as a part of the host's mechanism for defense against microbial invasion.

The manner in which erythrocytes enhance phagocytosis of bacteria by circulating phagocytes was exemplified in a film prepared with the collaboration of Dr. R. Robineau at Hospital St. Antoine in Paris. The chief features of the film may be summarized as follows:

1. A washed suspension of *Micrococcus aureus* was added to a suspension of freshly collected human blood which had been defibrinated by shaking with glass beads. Within 15 minutes greater than 90 per cent of the bacteria had become adherent to the erythrocytes. This reaction has been termed "immune-adherence" since related experiments have shown that both antibody against the bacterium and complement are essential as sensitizing substances. Further, only erythrocytes from man and monkey react to any significant extent with a wide variety of presensitized micro-organisms, e.g., starch granules, Diplococci, Salmonella and Shigella, Mycobacteria, Rickettsia, and certain viruses. Finally, evidence has been obtained which suggests that immune-adherence takes place *in vivo* in monkeys and therefore by analogy in human beings.

2. Observations were made of phagocytosis of the adherent bacteria. The ease with which the phagocytes engulfed the adherent staphylococci was remarkable when compared with engulfment of single or agglutinated bacteria which were floating free in plasma.
3. There was no phagocytosis of erythrocytes.
4. Numerous examples were noted of two or more erythrocytes attached to one bacterium or to a small clump of bacteria. This clumping of erythrocytes has since been examined in detail and a technique developed for simple measurements of immune-adherence by visual observations of "agglutination" of the indicator erythrocytes. This technical development has been particularly useful in assays for immune-adherence with viruses.

Immune-adherence would appear to have two main applications: (a) in immunological assays *in vitro* for antibody to a variety of particulate antigens including viruses, and (b) in studies designed to demonstrate the sequence of events which are induced upon invasion of the human host by bacteria and perhaps by viruses. As yet no definitive studies have been performed upon factors or disease states which might adversely affect the reactivity of the erythrocyte as an opsonin. It would seem, however, that the red cell may be added to the list of substances which should be studied, particularly in those disease syndromes which are characterized by a high susceptibility to bacterial or viral infection.

M. Lotz

ANATOMY SEMINAR

November 13, 1956

FACTORS INFLUENCING THE MOVEMENT OF THE POLYMORPHONUCLEAR LEUCOCYTE. By M. M. Lotz (from the Sir Wm. Dunn School of Pathology, Oxford, England).

The remarkable ability of the polymorphonuclear leucocyte to exhibit chemotaxis and phagocytosis is a phenomenon that has been extensively studied, intensively investigated, but, on the whole, little understood. It had been reported that calcium, serum, and complement were necessary for chemotaxis to occur in leucocytes, and that substances such as citrate, azide, fluoride, and cyanide could abolish the chemotactic response without impairing the motility of the cell.

In the present work some of the factors that are alleged to influence chemotaxis and phagocytosis were investigated. The method used for studying chemotaxis and phagocytosis was a photographic trace technique in which human polymorphonuclear leucocytes, isolated from a drop of blood obtained by a finger prick, were allowed to move between a slide and coverslip and their movement relative to a test object was recorded photographically. A chemotactic response was shown by a polarization of the traces made by the leucocytes in the vicinity of the test object which was a small clump of *Staph. albus.*

Experimental results indicate that the polymorphonuclear leucocyte can chemotax and phagocytose in solutions devoid of complement or serum, or in media with very low concentrations of calcium and magnesium to which

sizable amounts (15 mg%) of ethylenediaminetetracetic acid were added in order to chelate divalent ion present as impurity. The ability of this cell to remain motile for as long as four hours in solutions devoid of glucose protein, fatty acids, or any available energy supply suggests that the cell has an intrinsic energy store, presumably glycogen. It is not possible to suppress chemotaxis in the polymorph by using enzyme inhibitors such as fluoride, azide, cyanide, iodoacetate, or pyrophosphate. Chemotaxis is suppressed only when motility is suppressed, that is, under conditions toxic to the cell.

The ability of the polymorphonuclear leucocyte to chemotax and phagocytose in solutions containing 0.011 molar sodium cyanide and 0.005 molar sodium fluoride suggests that this cell's survival is not acutely dependent on an oxygen supply. Further studies on the oxygen requirement using the new micro-electrode apparatus for oxygen determination devised by Harris indicate the following interesting features: The oxygen uptake of this cell is independent of the oxygen tension of the ambient medium; even in media containing very high concentrations of oxygen, the polymorph utilizes primarily (*ca.* 90 per cent) the anaerobic (Embden Meyerhof) mechanism to metabolize glucose; in media with no measurable concentration of oxygen the polymorph remains viable for periods of time similar to its viability in aerobic media. *It is concluded that,* in all probability, the polymorphonuclear leucocyte is a facultative anaerobe. Convincing proof rests, of course, on a demonstration of a "Pasteur Effect" which, because of technical difficulties, has not yet been demonstrated with desired accuracy. Intuitively, however, it seems unlikely that one could explain this prolonged anaerobic viability by assuming that the cell is acquiring an oxygen debt.

Additional studies indicate that the polymorph can withstand decreases in tonicity of up to 125 milliosmoles and increases in tonicity of up to 500 milliosmoles when these increases are produced by substances, such as urea, which enter the cell. When hypertonicity is produced by substances which do not enter the cell, such as mannitol, sucrose, or sodium chloride, increases of 125 milliosmoles can be withstood. Finally, repeated studies indicate that the phenomenon of negative chemotaxis is as yet unsupported.

M. M. L.

PHI DELTA EPSILON LECTURE

November 28, 1956

TISSUE TRANSPLANTATION. By Gustave Dammin, Pathologist-in-chief, Peter Bent Brigham Hospital, Boston, Massachusetts.

It has been noted that the same relation exists between kidney, skin, spleen, lung, liver, and other organs with a large amount of stroma inasmuch as they are able to be homo-transplanted. It is believed that there is an antigenic relation between them and that homografts are rejected on an immune basis. Kidney disorders often necessitate transplantation, so this organ has been studied as well as skin since the latter acts like renal tissue and is more easily handled.

The uremic state has been studied as a possible altered immune state and this was the common denominator in most of the cases used. Differences in blood groups and sex had no effect on the survival of grafts. Renal homografts which were rejected exhibited progressive loss of tubular lumina, appearance of red blood cells in glomeruli, and infiltration of plasma cells and lymphocytes around glomeruli and small vessels, producing an arteritis suggestive of an immune response. Kidneys which were accepted for any length of time showed normal glomeruli and tubules spread somewhat by edema fluid. Occasionally, atherosclerotic changes were seen throughout the body after kidney homografts.

Skin grafts were performed on the anterior surface of the forearm so that they could easily be examined using a stereoscopic microscope. Periodic biopsies were also performed. Homograft of skin to a normal person exhibited petechiae and sloughing within nineteen days. An autograft and homografts with and without cortisone were received well by a uremic patient. Patches which were rejected by patients showed degeneration and absence of elastic fibers in the interval between the graft and the recipient bed. The absence of smooth muscle is also a sign of rejection. Before destruction of a graft there is an infiltration of plasma cells, lymphocytes, and some eosinophils, and blockage of this infiltration by means of a filter can delay the rejection. Uremic and agamma-globulinemia patients are capable of the latter type of cellular response, but grafts persist in these individuals, indicating that there is some alteration of the immune response to allow acceptance. This is the case in burn patients as well where a survival of dermis and epidermis is seen. These people are capable of cellular response and antibody production, and if one cannot find a fault in this, perhaps it is due to a reduced reactivity to the antigen-antibody complex. Complement level and alterations in properidin are in the process of being studied with the hope that more information may be gained in this regard.

J. A. O'NEILL

NU SIGMA NU LECTURE

December 4, 1956

AUTO-IMMUNE HEMATOLOGIC DISORDERS. By William Dameshek, Director, Blood Research Laboratory, New England Medical Center, Boston, Massachusetts.

The study of immunological hematology may be divided into several stages. First was the era of basic preliminary studies, including the discovery of "alexin" by Bordet and an isohemolysin in goats by Ehrlich. Then came the discovery of blood groups by Landsteiner, an autohemolysin in paroxysmal cold hemoglobinuria by Landsteiner and Donath, hemolysins in hemolytic anemia, auto-agglutinins in acquired hemolytic anemia, hemolytic splenomegaly, and therapeutic splenectomy, etc. About 1937, Schwartz and Danesh showed in two of three cases of a peculiar poikilocytic anemia that unheated (56°/hr.) serum lysed RBC's of all groups and that when the anemia improved, the hemolysin disappeared. Subsequently, experimental

immunohemolytic anemias were produced in laboratory animals. Increased hypotonic fragility and spherocytosis were characteristic. A period of rapid development followed in which Rh factor and isohemolytic disease, new methods for antibody detection, etc., were studied.

There are two types of hemolytic anemias, congenital, carrying an intrinsic defect, and acquired. RBC's normally undergo destruction in the body at a constant linear rate. If a defect-producing increased fragility is present, this rate is exponential. The concept of acquired autohemolysis envisions RBC's with surface altered by chemicals, infection, etc., and not "recognizable" to antibody-producing elements. Cells weakened by the concomitant attack of antibody undergo lysis at an increased rate due to physical trauma, trapping by the spleen, etc.

Therapy for iso-immune anemia (Rh) is exchange transfusion, as Ab is only present via passive transfer. Conversely, auto-immune anemias are self-perpetuating. Primary therapy is directed against the R.E. system (HN_2, ACTH, Steroids). Thus, good results were seen in 37 of the first 40 cases treated. In only 8 cases was splenectomy required.

It has recently been demonstrated that plasma from idiopathic thrombocytopenic purpura patients injected into normals reduces the platelet count. Similarly, after multiple platelet transfusions, survival time is progressively shortened. In certain cases quinidine has been implicated as a haptan. It is suggested that thrombotic thrombocytopenic purpura and lupus erythematosis may be immunologic disorders.

N. R. MORRIS

NEUROLOGICAL STUDY UNIT

December 4, 1956

NEW DEVELOPMENTS IN NEUROCHEMISTRY. By David Nachmansohn, Associate Professor of Neurology, College of Physicians and Surgeons, Columbia University, New York.

Early formulations of the hypothesis of neurohumoral transmission held that acetylcholine (or epinephrine) was released at the synapse and acted as an intercellular "messenger" molecule across the synapse. Yet this junctional mechanism bore little relation to the known changes in membrane polarity and ion permeability associated with impulse propagation along the axon. In recent years, however, Dr. Nachmansohn and his co-workers have formulated and established a modified humoral theory which gives hope of integrating the observed polarity changes, ion movements, and molecular events in impulse propagation and transmission.

In the new conceptual scheme the action of acetylcholine is not an inter- but an intracellular process occurring within the conducting membrane. The action of acetylcholine is believed to be mediated by four protein molecules in the membrane. Two of these proteins, acetylcholinesterase and choline acetylase, are well known and widely studied enzymes, whereas relatively little is known of the structure and properties of the other two, the acetylcholine storage protein and the acetylcholine receptor protein. The change in membrane permeability during conduction may be caused by a

change in the configuration of the receptor protein molecule as it binds acetylcholine. All four proteins, as they react with acetylcholine, are believed to hold the molecule temporarily at similar binding sites. This site consists of an anionic portion which binds the cationic nitrogenous end, and an esteratic portion which binds the ester group of the acetylcholine molecule.

Dr. Nachmansohn's recent work on the four protein system is of great theoretical and practical interest. The deadly alkyl phosphate "nerve gases," such as DFP (diisopropyl fluorophosphate) are known to act by inhibiting acetylcholinesterase. Apparently, DFP blocks the enzyme by firmly phosphorylating it at the esteratic site which, normally, binds the acetyl group of acetylcholine. Working from this hypothesis, a compound, PAM (2-pyridine aldoxime methiodide), was designed and produced to occupy the binding site, displace the alkyl phosphate (DFP), and break it free from the enzyme molecule. PAM, though it vigorously displaces DFP, is itself rapidly metabolized off from the binding site, leaving the way clear for normal activity. Reactivation of acetylcholinesterase with PAM causes a dramatic, life-saving reversal of DFP poisoning in experimental animals. Although it has not yet been tested for human use, PAM gives every promise of being an effective antidote for military use and for the increasingly numerous cases of accidental DFP poisoning of agricultural workers who use it as an insecticide.

ROGER P. ATWOOD

December 7, 1956

FOLLOW-UP STUDIES IN MITRAL VALVULOTOMY. By Professor Kenneth Donald. University of Birmingham, England.

A small series of cases of young adults was studied preoperatively, and six months and two years postoperatively with respect to clinical improvement and cardiac and respiratory dynamics. Cases were evaluated by three independent clinical interviews and their disability graded. Cardiac catheterization and ventilatory studies were used to assess cardiorespiratory function.

Of the 28 patients who completed the study, in 9 there was considered to be clinically excellent results, 11 very good, 5 good, and 3 poor or unimproved by the operation. Of the 10 patients who had had one or more episodes of failure, 5 were judged to show excellent results, 3 good, and the remaining 2 were not helped.

Oxygen uptake and pulmonary ventilation fell to normal levels in the first postoperative study. Pulmonary arterial and pulmonary capillary pressure were also decreased at this time. At the two-year follow-up there was a general tendency for these values to rise above the levels found at the end of six months, and pulmonary arterial pressure in exercise was found to rise to abnormal levels. The cardiac index was found to be lowered in the postoperative studies. The paradox of the persistence of the clinical status and the early deterioration of the cardiorespiratory function is not yet explained.

ROBERT NEUWIRTH

BIOPHYSICS-ZOOLOGY SEMINAR

January 10, 1957

THE TIME OF SYNTHESIS OF DESOXYRIBONUCLEIC ACID: TRACING LABELED CHROMOSOMES THROUGH CELL DIVISION. By J. Herbert Taylor, Professor of Botany, Columbia University, New York, New York.

Little has been added to our knowledge of chromosome structure since the early 30's when Belling postulated that the chromosome consisted of a group of 2500 chromomeres and proposed a model for duplication and crossing over. A significant advance was made in 1948 when Vendrely and Vendrely found that the amount of desoxyribonucleic acid was equal in similar nuclei, that an average amount of DNA is present in the cells of the same tissues, and that cells of diploid somatic tissues had twice the amount in haploid sperm.

Two techniques are available for the detection of DNA in cells, photometric measurements (Caspersson) and Feulgen dye (Porter). Combining phosphorous labeling with these methods has shown that the DNA in a cell is increased during interphase (eight hours before metaphase, the time varying in different tissues) rather than being laid down at a constant rate during the whole division cycle of a cell. The amount of incorporated labeled material may be measured by counting the developed "grains" on an autoradiographically exposed emulsion layer over the fixed and stained cells. An advantage in the method is that the pattern of the grain image may be compared directly with the underlying Feulgen-stained chromosomes. DNA is turned over at such a slow rate in the cell that the labeled DNA will remain with the same nucleus. Mazia and Plaut reported that when carbon-labeled chromosomes are duplicated, one set of the daughted chromosomes is labeled, the other not. Unfortunately, however, the carbon label resolution on film is not good and the grains are scattered on the image.

In the present study made at the Brookhaven Laboratories, tritium was used for labeling since the low-energy beta particle gives good resolution in the emulsion. Working with very active material (3000 μc/ml), growing roots of broad bean, *Vicia faba*, were left in a thymidine solution for eight hours. (Thymidine was used since the tissues normally contain no thymidine and therefore it is utilized immediately in DNA synthesis.) The roots were then put in colchicine to allow nuclear reduplication with arrested cell division. Polyploid nuclei resulted, tetraploids after one DNA synthesis and octoploids after two. Following the first chromosomal division (synthesis from labeled precursor), both daughter chromosomes are labeled, but after going through a reduplication cycle without the labeled precursor, one of the daughter chromosomes is labeled, the other not. The fact that some of the chromosomes showed both radioactive and inert areas was attributed to sister-strand exchanges. The frequency of exchange in some of the tissues was as great as one per chromosome per division cycle. No chromosome breakage was noted although the cells had taken up large amounts of labeled tritium.

This evidence of DNA synthesis supports as a theoretical chromosomal model, a ribbon in which the edges and center contract at different rates in order to permit coiling into a tube-shaped helix. The core is undefined material, with the outer edges consisting of DNA threads attached to it.

Labeled thymidine and colchicine experiments have been carried out in addition on two liliaceous plants and *Crepis* with approximately the same results.

GEORGE E. WATSON

BIOPHYSICS DEPARTMENT, BOTANY DEPARTMENT, AND
ZOOLOGICAL JOURNAL CLUB

January 23, 1957

HETEROCHROMATIN AND THE PROBLEM OF NUCLEAR DIFFERENTIATION. By Jack Schultz, Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania.

Although the concept of nuclear differentiation is clouded by semantics, morphological changes in chromosomes during development have been demonstrated. Alterations in the economy of the cell are correlated with activities in the heterochromatic regions of the chromosomes.

Previous experiments with Caspersson suggested that heterochromatin played an important part in nucleic acid synthesis. Genetic investigation using the Y chromosome as a typcial heterochromatic region is complicated because accurate mapping has not been possible. No good markers are available, genetic recombination is hard to follow, and the position effect in that region is most "lurid." Freed has studied the behavior of the Y chromosome in the nurse cells of Drosophila. He has shown that although the nurse cells increase in size during development, the Y component does not; it remains about the same size as in the far smaller follicles. DNA measurements are correlated with increase in the ploidy of cells in the series of duplications, up to the 512-ploid condition of the nurse cell. Microphotometry reveals that more DNA is present in the XXY nurse cell than in the XX, and that this amount is in excess of what the Y alone contains; while that of the salivary gland is not as great. Since the nurse cells function to synthesize DNA while the salivaries secrete a mucoprotein at pupation, the implications are that the heterochromatin is more active in DNA-synthesizing cells.

Chemical measurements of RNA reveal no differences in the amounts in XX and XXY salivaries, but Levinbook and Travaglini have found an excess of the adenine component in the XXY nuclei of oocytes. Chromatography has further demonstrated that more cytosine is present in XX than in XXY nuclei while the reverse is true for thymidine. The Y chromosome therefore influences the course of RNA synthesis. The genotype of the Drosophila has no effect on the total amount of nucleic acid present.

Investigations have also been carried out on the heterochromatin-induced variegations in Drosophila. The variegation due to a transposition of

euchromatin in the white locus to a heterochromatic region of another chromosome affects the eye, Malpighian tubules, and salivary glands. The effects are also sensitive to environmental change. Amethopterin added to the culture medium increases the production of variegated regions (stubble bristle over wild type). The effect is reversed when thymidine is combined with amethopterin, but thymidine alone produces no change. Deoxyuridine has no effect, while deoxyadenosine reduces variegation sharply.

This information further strengthens the widely accepted concept of a chromosome as the unit of nucleic acid synthesis.

GEORGE E. WATSON

MICROBIOLOGY SEMINAR

February 1, 1957

RECENT STUDIES IN THE VIRUS ETIOLOGY OF MOUSE LEUKEMIA. By Charlotte Friend, Sloan-Kettering Institute, New York, New York.

Leukemia can be transmitted from one mouse to another by a cell-free agent. This result, known since 1946 for transmission of leukemia to newborn mice, has now been extended to transmission of tumor to adult mice.

Discovery of small virus-like inclusion bodies in the cytoplasm of cells of the Ehrlich ascites tumor of a strain of Swiss mice (a noninbred strain) suggested the possibility of virus etiology of this tumor. Cell-free extracts of the tumor were given to adult Swiss mice. After a latent period of two weeks, a disease developed which was characterized by an enlarged palpable spleen, a chronic course of 30 to 100 days, and overt illness several days before death. The white blood cell count rose sharply, sometimes reaching 300,000 terminally (normal—16,000), while the red blood cell count fell to anemic levels. Large, fragile mononuclear cells, probably of granulocytic origin, dominated the peripheral blood. Some nucleated red cells appeared in the terminal stages.

Post-mortem findings included a greatly enlarged spleen and liver and slightly enlarged lymph nodes. The bone marrow was almost completely replaced by the abnormal mononuclear cells with enlarged nuclei and thin rims of cytoplasm. Pathological changes were also found in the lungs, heart, and other organs.

The disease has been propagated by serial transfer of cellular suspensions and cell-free filtrates of affected organs. It has not been possible, however, to re-isolate the agent from the ascites tumor itself.

Neutralizing antibodies were obtained from rabbits immunized with tumor extract. These antibodies did not cross-react with the virus of lymphocytic choriomeningitis.

At present, work is in progress on several additional aspects of the problem. Tissue culture, chemical purification, and electron microscopy of the agent itself should yield further information on its exact nature. In addition, there is an active search for effective chemotherapeutic agents.

LISA A. STEINER

THE COMPARATIVE ENDOCRINOLOGY OF VERTEBRATES. Part I. *The Comparative Physiology of Reproduction and the Effect of Sex Hormones in Vertebrates.* Memoirs of the Society for Endocrinology No. 4. I. C. Jones and P. Eckstein, Eds. Cambridge, Cambridge University Press, 1955. x, 253 pp. $8.50.

This volume presents, in 14 chapters by different authors, the formal papers presented at a symposium held at the University of Liverpool in July 1954. The title of the book is taken from the sub-title of the symposia on comparative endocrinology of vertebrates. Each chapter is followed by a page or so of discussion so almost all of the 58 participants at the symposium contributed. Each chapter is followed by a list of references that seem well selected to illustrate major points. No effort has been made to treat the subject in an encyclopedic manner. More extensive review articles are cited to cover general areas of background observation. The format is attractive and readable.

The first 113 pages (6 chapters) cover the descriptive aspects of the physiology of reproduction in teleosts, amphibians, reptiles, and birds. One chapter on reproduction in mammals follows. These chapters describe the reproductive phenomena, influences of environmental factors, and variations observed among animals of different species within the orders.

A chapter on the evolution of viviparity in vertebrates, "that has been evolved independently many times," is followed by chapters in which results of manipulative experiments are reported and which follow the same outline as the descriptive chapters. In general it is stressed that "endocrine evolution is not an evolution of hormones but an evolution of uses to which they are put. . . ." Ontogenic sequences in mammals are also reported in one chapter. Each author is not only a student of but an outstanding investigator in his area.

Knowledge of comparative endocrinology of reproduction of vertebrates is strikingly limited. The diversity of anatomical, physiological, and behavioral modification associated with the two-organism integrations necessary in vertebrate reproduction is understood reasonably well in only a few species; "there are 20,000 species of teleosts, only fifty of them, belonging to ten different orders have been studied." The tendency for concern primarily with reproduction in mammals or commercially utilizable birds or fishes, as well as the complexity of structural, functional, and behavioral qualities that require the participation of two separate individuals has limited the expansion of knowledge of reproductive physiology. The symposium reported in this small volume points out much of what is known of the comparative physiology of reproduction but equally as well those areas where the unknown and assumed blend with the observed, interpreted, and known. The problems have been studied "by the building up of an ever-increasing fund of information into a supposedly even more complete picture of how an animal works—an endless job of integrating constantly changing ideas. Others . . . are more interested in breaking down the problem into bits, which can be investigated piecemeal in a simple way. . . ."

The symposium was well planned. Biologists, zoologists, veterinarians, anatomists, and physiologists attended, among them outstanding investigators from Britain, Canada, France, Holland, and United States. The report can be criticized most because it is not more extensive.

WILLIAM U. GARDNER

SCIENCE AND CIVILIZATION IN CHINA. By Joseph Needham. Cambridge, Cambridge University Press, 1956. Volume II, xxii, 696 pp. $14.50.

In this second volume of Professor Needham's proposed eight-volume treatise on Chinese science and civilization, he describes in extensive and loving detail the philosophical background against which the specific sciences in China developed. In the first part, Confucianism, Taoism, Mohism and Legalism are analyzed with special attention being paid to the implications of these philosophies for what can best be described as an attitude towards nature. It is this attitude towards nature which Professor Needham sees as a basic element which tends to determine the vitality of science in any culture.

After revealing the basic philosophical systems, Professor Needham turns to the more specifically scientific questions of definition of elements, cosmological and cosmogonical speculation, concepts of natural law, matter and energy, tracing the evolution of these concepts, not only as self-contained ideas whose changing properties follow certain logical principles, but also as reflections of the evolution and development of Chinese society itself.

The volume is completed by an extensive bibliography and index, and the whole beautifully bound and printed by the Cambridge University Press.

There is little that one can say in the presence of such a magisterial volume. Professor Needham has obvious command of his sources and weaves them together into a coherent, logical, and literate whole. His sense of historical reality is acute; his analysis of philosophical systems is keen and the ability with which he reveals the interconnections of Chinese philosophical and scientific thought is admirable. This is, in short, a volume which every student of the development of man will treasure. Furthermore, it makes one even more impatient for the appearance of the succeeding volumes of this great work.

L. PEARCE WILLIAMS

THE ORGANIZATION OF THE CEREBRAL CORTEX. By D. A. Sholl. New York, John Wiley and Sons, Inc., 1956. xvi, 125 pp., 12 plates. $4.25.

Understanding of the functions of the brain is a subject that requires the integration of different disciplines, and the overlapping among them constitutes a fruitful field for speculation and research. One of the virtues of this book is that the author has tried to correlate the recent work of anatomists, clinicians, physiologists, and psychologists. Qualitative and quantitative information concerning histology of the cerebral cortex is discussed, emphasizing the individual variations, the disagreement of cortical maps and cortical instability to electrical stimulation. The functional im-

portance of the complexity and pattern of neuronal connections was emphasized by Cajal long ago, but since the determination of the number of connections of an individual neuron is very difficult, Dr. Sholl's stimulating idea is that "the connective field of the neurons may be defined and measured in statistical terms." The background of the author led him to adopt a statistical approach to the problem. By this means some explanation can be offered for the plasticity of response shown in physiological and psychological experiments. The exposition is extremely lucid, but some arguments against the thesis may have been underestimated. For example, it is true that localization of some functions such as "memory" cannot be pinpointed and regional specialization is sometimes difficult to show. However, the problem could be dealt with in terms of *regional hierarchies,* in the sense that some areas play a more important rôle in controlling a specific function. Memory, for instance, is impaired by temporal lobe destruction but is not modified by extirpation of area 4, and it is difficult to accept "memory" as a "general attribute of all cortical tissue."

"Cortical instability" cannot be considered axiomatic. There is some feeling in the literature that it could be attributed to artefact if experimental conditions were changed, and particularly if the electrodes to stimulate the cortex were reapplied. Another questionable point is the concept of the cortex as the highest part of the central nervous system; the importance of "subcortical" neuronal fields in the highest mental processes should be considered.

However, these are questions occasioned by the excellent intellectual level of the book which will probably stimulate many constructive controversies. In the last chapter mathematical theories of cortical organization are discussed and a main thesis is expressed: models used to study the cortex "should be based on the concept of probability and discussed in a statistical language." Complicated problems are explained clearly but with depth, which makes reading of the book very enjoyable. Six pages of bibliography are offered and alphabetical indices of subjects and authors complete the book.

JOSÉ M. R. DELGADO

CLINICAL CHEMISTRY. Principles and Procedures. By Joseph S. Annino. Boston, Little, Brown and Co., 1956. 280 pp. $7.50.

This book fills a long felt need for a text which can be recommended to those who enter the hospital chemistry laboratory to undertake practical technical work for the first time. Especially where the background has been theoretical alone, or is lacking in the basic sciences, there has been no single volume which served as a satisfactory introductory reference.

The author has defined in a clear, well-organized manner fundamental subjects and basic principles with many details heretofore not described, but which are indispensable to the successful performance of work in this field. The graphs, tables, and excellent illustrations add to its value. Part one deals with apparatus, solutions, colorimetry, a review of quantitative analysis, information concerning the collection, preservation, and identifica-

tion of samples and normal values. Part two is devoted to methods of analysis of biological fluids. The description of each method includes an introduction, recommended procedure or procedures, discussion, brief note on the physiological significance, and bibliography.

The subject matter is treated in a simple and direct way, which is by no means complete, but the material is well selected to be representative and significant. Directors of hospital laboratories, clinical pathologists, teachers of courses in medical technology, and others who have responsibility for technical training in either the research or service laboratory will find it a useful aid.

PAULINE M. HALD

AGEING IN TRANSIENT TISSUES. Volume 2, Ciba Foundation Colloquia on ageing. G. E. W. Wolstenholme and Elaine C. P. Millar, Eds. Boston, Little, Brown and Company, 1956. xi, 263 pp. 96 illus. $6.75.

One is accustomed to think of ageing in terms of the whole organism; it is stimulating to consider the much briefer life span of erythrocyte or placenta. The tale of man's life cycle could be the composite account of his tissues and cells, the records of the growth, deeds, reproduction, and death of the generations of a strange and varied population. Some of these histories are considered in Volume 2 of the *Colloquia on Ageing*. As is the practice in the outstandingly useful meetings at the Ciba Foundation, a small group of investigators (in this case, twenty-seven European and American scientists) gathered to present and discuss reports. What they had to say about *Ageing in Transient Tissues* is the substance of this volume.

Topics include the fetal reproductive system (*in vitro*) and fetal endocrinology, germ cells, and ovarian tissues. The descriptions of the ageing of the placenta alone make a story sufficiently outstanding to justify the publication of the book, and there are other important reports. Wislocki's paper on "The Growth Cycle of Deer Antlers" is fascinating. Also described is the ageing of mitochondria, axillary apocrine sweat glands, leaves, and red cells. This reviewer found the papers of Dempsey (mitochondria), Fawcett and Burgos (male germinal and interstitial cells), Villee (biochemical aspects of placental ageing), and Tuchmann-Duplessis and Mercier-Parot (effects of administration of growth hormone and cortisone to fetal rats) to be particularly clear and helpful. Several reports are illustrated by fine photographs taken with both light and electron microscopes, and there are numerous graphs and tables. Author and subject indices and skillful editing add further value to an excellent and useful volume.

THOMAS R. FORBES

HORMONES AND THE AGING PROCESS. Earl T. Engle and Gregory Pincus, Eds. New York, Academic Press, 1956. 322 pp. $8.50.

This series of papers represents the proceedings of a conference concerned with *Hormones and the Aging Process* in the broadest sense, embracing as it does the endocrine aspects of cardiovascular and neoplastic diseases, diseases of increasing prominence in an aging population. The contributions

range from detailed reports of animal experiments and of metabolic balance studies in man to a very stimulating and speculative essay by D. J. Ingle concerning Selye's adaptation hypothesis.

The papers in this symposium bring out very clearly that although intensive studies have been carried out in certain spheres concerning the effects of aging, there are many other areas which are virtually untouched. The papers of Pincus and of Samuels, for example, delineate the meticulous and detailed work which they and their coworkers have done in the study of the effects of age upon steroid metabolism. In contrast, Rawson's review of the available data concerning the thyroid in the aging process is valuable in laying bare how very meager are the facts presently at hand and in emphasizing the many important problems in this field which recently developed research techniques have now opened for productive exploitation.

This well-edited conference series constitutes an excellent review of current research and thinking in a number of areas of active endocrine investigation.

PAUL MANDELSTAM

BONE STRUCTURE AND METABOLISM. Ciba Foundation Symposium. Boston, Little, Brown & Company. 292 pp. $8.00.

This well-bound and well-printed volume covers the results of a conference by various men from a good part of the world who are doing fundamental research on bone, its structure, and its many functions. A complete review of the subject is, of course, not within the scope of such a conference nor is it claimed to be such. Sections of the book are devoted to structure, from the anatomical to the molecular level, of the organic and inorganic components. There are sections on remodeling, the mucopolysaccharides, the time relationships in matrix formations, the behavior of radioactive sulphur, phosphate and calcium in normal bone, growing bone, and in fractures; on bone citrates and magnesium; a rather brief section on the dynamics of bone nutrition; and sections on vitamin D, parathormone, vascularity, the localization of advantitious radioactive substances; and the discussion of several new diseases related to vitamin D physiology.

The book will be of considerable interest to people who already have some knowledge of the fundamental aspects of bone and are, to some extent, acquainted with the research that has been done over the last twenty years. The novice in this field will find the material rather tough going.

Throughout the whole presentation an excellent idea is gained of the various means by which the subject is being attacked in various laboratories and of the individual who is interested in bone research; many possible new approaches become evident during perusal of the material. At the conclusion of each section, which is in the nature of a formal paper, there is included some discussion by the members of the conference, and the notes from this discussion are in some instances as valuable or more so than the material in the paper. Each section is accompanied by a very useful bibliography, the majority of the titles being in the English language or available in translation.

There are a number of illustrations and figures which, on the whole, are quite well selected and of good quality. In some instances, the material presented happens to represent an area over which considerable controversy exists at present and, unfortunately, both sides of the controversy do not receive the same treatment. A student perusing this book should be aware of this so that he may keep an open mind. It would, of course, be beyond the scope of such a conference to try to present all sides of the subject completely, for the book would then become unwieldy in size.

On the whole, this is a very useful and informative study which provides and conveys a good impression of the current work being done on the subjects covered.

HAROLD M. FROST

BIOLOGY OF THE LABORATORY MOUSE. Staff of the Roscoe B. Jackson Memorial Laboratory. George D. Snell, Ed. New York, Dover, 1956. viii, 497 pp., illus. $6.00.

This book "is an unabridged and unaltered reprint of the first edition." The first edition was originally published by the Blakiston Company in 1941 and has been out of print for some time.

Since this book was first published, an additional wealth of information has been added to the literature. The mouse has invaded new fields of research and is currently utilized in the fields of biology, biochemistry, embryology, virology, bacteriology, endocrinology, genetics, cancer research, and in radiation studies. It would be highly desirable if a new edition of this book were to be published and were to incorporate the findings since 1941. Nevertheless, it is to the advantage of all workers in research utilizing mice to have this edition in print once more, because it contains a great deal of valuable basic information. This book has had wide circulation and was extremely popular with research personnel, and the fact that this is a reprint rather than a new edition should not detract greatly from its popularity.

A minor criticism is that the quality of the paper is not up to that used in the original printing, but for the sake of economy it may be warranted.

MERTON S. HONEYMAN

PERSPECTIVES AND HORIZONS IN MICROBIOLOGY. Selman A. Waksman, Ed. New Brunswick, Rutgers University Press, 1955. x, 220 pp. $3.50.

The contributions included in this volume are based on papers presented on the occasion of the dedication of the Institute of Microbiology at Rutgers University. The thirteen papers are grouped under the general topics of The Microbe as a Living System, Metabolism of Microorganisms, and Microorganisms and Higher Forms of Life. Since the invited participants are prominent microbiologists, the book can hardly fail to contain critical and informative presentations which are well worth reading. However, the great number of symposium volumes which have appeared in recent years, and which will undoubtedly continue to appear in the future, compel the

reviewer to subject this volume to a more critical scrutiny than might otherwise be justified.

The purpose of a review or a symposium includes the presentation of a summary of the history and of the current knowledge of a particular subject for the benefit of the nonspecialist, and the exposition of a considered critique, experimental philosophy, or other generalization for the benefit of both specialists and nonspecialists alike. The appearance of many reviews and symposia, however, confuses the prime purpose, namely, the presentation of a survey covering a large body of literature. The reviewer feels, therefore, that in evaluating a symposium volume technical competence alone is not a sufficient and adequate criterion, but that special virtues must be exhibited by the book to make it a worthwhile contribution. Among the papers found in the present volume those by Barker, Lederberg, and Wilson meet these special requirements: they are impressive because they combine unusual clarity and critical evaluation with the highest technical competence. This judgment is meant in no way to detract from the value of the other chapters, but only to emphasize the extreme readability of the three papers cited above.

In general, this informative book can be recommended to graduate students as a survey of several important topics in microbiology. It is an exaggeration, however, to claim that it represents "a milestone in the history of a science," as the editor remarks in the introduction. Perhaps the word "milestone" should be reserved for symposia such as the *Currents in Biochemical Research* which appear at intervals of ten years, or for symposia which present material not otherwise available and which have an immediate stimulatory effect on research such as the volumes edited at the McCollum-Pratt Institute. The many smaller, occasional symposia which frequently duplicate each other are a dime a dozen. This particular book suffers in addition by the inclusion of three speeches, including the editor's own, for which the editor claims "historical interest." These speeches may make pleasant reading at the moment, but are neither informative nor inspirational.

These mild shortcomings do not detract from the general value of the contributions. The book is attractively bound, printed, and, a rarity nowadays, moderately priced.

WOLF VISHNIAC

CELLULAR MECHANISMS IN DIFFERENTIATION AND GROWTH. Dorothea Rudnick, Ed. Princeton, New Jersey, Princeton University Press, 1956. vii, 236 pp. 34 plates. $7.50.

Each year sees the publication of another group of papers from the Growth Symposia of the Society for the Study of Development and Growth. This, the fourteenth, and again under the editorship of Dorothea Rudnick, is of no less interest than the stimulating volume of the previous year.

The current selections have been divided into two broad categories. Included under intracellular events are analyses of phototropic responses in *Phycomyces* by Delbrueck and Reichardt, who develop a model which ex-

presses sporangiophore growth, and of the morphologic aspects of nuclear RNA. This latter paper, by a group from the University of Chicago, includes unusually clear electron photomicrographic evidence in support of the authors' claims. The structure of plant cilia and their similarity to those of animals (Manton) and a study of nucleo-cytoplasmic interactions and morphogenesis in *Stentor* (Tartar) are the contributions concluding this section.

A greater degree of unity can be detected in the second part, which is comprised of papers dealing with intercellular events. The reciprocal effects of humoral, neural, and other agents in neurogenesis are discussed by Hamburger, while Niu contributes a description of his technique of embryonic induction via diffusible substances. The Lederbergs deal with problems of transduction and consider some of the broader implications of bacterial genetics. The interactions of slime-mold cells in development (the Sussmans), an analysis of plant shoot growth (Wetmore), the induction of heterologous tolerance (Billingham), and a description of the differentiation of the myelin sheath (Geren) rounds out part two.

Altogether, the collection of Growth Symposium volumes represents an impressive indication of the progress being made in this difficult field.

PETER KLOPFER

CRYPTOCOCCOSIS. By M. L. Littman and Lorenz E. Zimmerman. New York, Grune and Stratton, 1956. 205 pp. $8.50.

This monograph is the first of a series to be written by the authors on systemic mycoses. It is an excellent review of the clinical aspects of cryptococcosis as well as the epidemiology, immunology, pathology, and treatment of the disease. Also included is a section on the isolation and identification of the etiologic agent, *Cryptococcus neoformans*. This fungus is not difficult to identify, but much care must be taken not to confuse it with encapsulated yeasts that are not pathogenic for man. The reader may be discouraged when he reads the chapter on Treatment and finds that there is still no compound available that promises a cure for cryptococcosis.

This volume is attractive, being printed on paper that has a glossy finish; it is well illustrated with many black and white prints but also contains four beautiful color plates. It is to be highly recommended to the internist, dermatologist, mycologist, and medical student.

LENORE D. HALEY

PRINCIPLES OF FUNGICIDAL ACTION. By James G. Horsfall. Waltham, Massachusetts, Chronica Botanica Company, 1956. 279 pp. $6.50.

The author of this excellent volume has been active for many years in the science of plant diseases and has contributed much to our knowledge of fungicides and their mode of action. The primary emphasis of this book is "on the mechanism of toxicity and on the types of compounds that produce effects."

Four main areas are covered: The techniques for studying actions of fungicides in the laboratory; the effect of fungicidal agents upon the metabolism of fungi; a discusion of various chemical compounds used in fungicides; and chemotherapy of plant diseases.

This book may be of limited interest to the physician but is highly recommended to the microbiologists and pharmacologists.

LENORE D. HALEY

THE BIOLOGIC EFFECTS OF TOBACCO. Ernest L. Wynder, Ed. Boston, Little, Brown and Company, 1956. 215 pp. $4.50.

This small volume consists essentially of six review articles and one essay. The first article, by Alvin I. Kosak, deals with the chemistry of tobacco smoke. This is a well-written and carefully documented section which emphasizes the need for more uniformity in experimental methods in the field and indicates a lack of knowledge in the important phase of aerosol retention. The chapter on pharmacology by Charles J. Kensler deals principally with the action of nicotine, although the possibility of other components of smoke being important is admitted. In the chapter on the cardiovascular system, Ellen McDevitt and Irving S. Wright conclude that the "use of tobacco by susceptible persons will result in the development and continued activity of thromboangiitis obliterans." They also state that although there is no evidence that tobacco is an etiologic factor in several other vascular diseases, including coronary artery disease, its continued use unfavorably influences the clinical course. The section on neoplastic diseases by Ernest L. Wynder presents the author's much publicized results and those of other workers interpreted to show the rôle of tobacco smoking in the causation of lung cancer. The review on the gastrointestinal tract by Robert C. Batterman "indicates only that our knowledge of the influence of use of tobacco upon the gastrointestinal tract is fragmentary." Doctor Batterman deplores the lack of scientific evidence available as to the relationship between tobacco smoking and the gastrointestinal tract. In the section on allergy, Francis C. Lowell concludes that skin sensitivity to tobacco is present in approximately one-third of the smoking population and less in nonsmokers, but true allergic manifestations to tobacco or tobacco smoke, such as dermatitis, asthma, or rhinitis are uncommon or rare. There is insufficient evidence to warrant the conclusion that vascular disease is caused by allergy to tobacco. The final chapter is on "Cause and Effect" by E. Cuyler Hammond. This deals with the statistical approach to epidemiological research.

Although this book contains numerous references to the literature on the various aspects covered, this alone is insufficient reason for publishing it. The material presented might be interesting as a point of departure for one planning to work in the field, but the paucity and weakness of conclusions based on scientific evidence suggest that this is a premature effort.

WILHELM S. ALBRINK

FOOD POISONING. By G. M. Dack. Chicago, The University of Chicago Press, 1956. xi, 251 pp. $6.00.

In this, the third edition of his well-known work, Dr. Dack has made a thorough revision and incorporated the newer knowledge of a subject which affects each of us at one time or another.

The first two chapters on chemical poisoning and poisonings due to substances found in plants, fish, and shellfish contain useful tables of specific agents, incubation periods, symptoms, and foods involved.

Botulism, staphylococcus food poisoning, and upsets caused by the multitudinous members of the Salmonella group of bacteria (really, infections rather than poisonings, as the author points out) are, of course, the best understood types and are discussed in satisfying detail. The space devoted to disturbances caused by *Streptococcus fecalis, Clostridium perfringens,* and certain other bacteria is necessarily limited because our knowledge of the rôle of these organisms is restricted. With this group, as with the Salmonellas, the process is primarily one of infection. In the concluding chapter, Dr. Dack lists those infections (the dysenteries, infectious hepatitis, trichinosis, those of viral etiology) which should not be confused with "food poisoning." He stresses the fact that symptomatology, epidemiology, and pathology are all essential in establishing a correct diagnosis and analysis of any outbreak. To this reviewer, however, the logic of including outbreaks due to the Salmonellas, for example, and barring those due to some other agents, is debatable: the classification seems to have its basis in custom alone.

Dr. Dack has brought together in concise fashion information on a problem which, as a result of our changing methods of food processing, is becoming increasingly important, as anyone who reads the weekly Communicable Disease Summary is aware.

PHILIP B. COWLES

HYPNOSIS AND ITS THERAPEUTIC APPLICATIONS. R. M. Dorcus, Ed. New York, McGraw-Hill, 1956. 313 pp. $7.50.

In the preface of this book the Editor describes how he collected the lectures of several participants in a course aimed at bringing together experimental, theoretical, and clinical aspects of hypnosis. This is the value and shortcoming of the book: it gives the viewpoints of eight writers, which in the lecture-course framework makes for breadth of view and wideness of the area covered but carries with it a lack of depth and cohesiveness. To write a book about hypnosis is difficult. We still do not know what hypnosis is, have difficulty in defining it in other than descriptive terms, and the literature is replete with contradictory findings, uncontrolled experiments, and inadequately reported procedures and data. One of the contributors to this book, Sarbin, stresses this point in his chapter on the physiological effects of hypnotic stimulation.

The first part of the book, theoretical and experimental, is a good but brief survey and discussion of most of the work in this area. For example,

the first chapter, "Theories of Hypnosis," covers this in twenty-eight pages. It gives the reader a good, rough orientation, and goes into detail in reporting the work of certain writers. It is a critical review in the good sense of that term. It is surprising, however, to find reference to only two papers and one book published since 1950, and to read that Kubie and Margolin's paper of 1944 is "the psychoanalytic school's final product up to the present time."

The chapters on clinical work with hypnosis have some of the same critical attitude that benefits the theoretical and experimental sections but they cover too much ground to treat the subject more than cursorily. In 124 pages the use of hypnosis in diagnosis, psychotherapy, general hospital medicine, and dentistry is described. A few cases, presented in detail, would be of greater benefit to the general medical reader.

It is this reviewer's opinion that the book gives a survey of most of the field, but it achieves this by, in most instances, skimming and rarely going deeply or coming to grips with many of the problems that the phenomenon of hypnosis raises. As such, its value seems to be that of a stimulus to reading and discussion by study groups.

RICHARD NEWMAN

NEW LIVES FOR OLD. By Margaret Mead. New York, William Morrow and Company, 1956. xxi, 548 pp. $6.75.

Margaret Mead is America's best known anthropologist and one of the science's genuine pioneers. In 1953, exactly 25 years after her first visit there, she returned to the Melanesian island of Manus because remarkable things had been happening to its people, and she wanted to understand better what had changed and how it had come about. Her findings are embodied in this interesting—at times, almost incredible—book, which belies its hackneyed title.

This is an attempt to record the transformation "of a people who have moved in 50 years from darkest savagery to the twentieth century." It gives us an inkling of how fast a society can change and purports to help us understand better by contrast those societies which are changing more slowly. Dr. Mead makes explicit an idea that many anthropologists have had in the backs of their minds for some time: that in certain situations, rapid change, if it affords to a people access to the whole institutional structure of a different society, may be socially more beneficial than slow change.

In 1928, Dr. Mead found the Manus fishing village she studied a setting for fierce adult competition, for involved and onerous economic manipulations designed to conciliate the household ghost rulers, and for a free and easy childhood terminated abruptly by the imposition of the loveless and demanding Manus standards of maturity.

In the years following, the people divested themselves of the rule of ghosts by becoming Catholics; young men going off to work for Europeans also helped to create change in the village, or at least to set the scene for such change. Then, in World War II, the Manus were inundated with Western culture, embodied not only in the artifacts of war and those

designed to make warring comfortable, but also in the behavior of Western troops—behavior this reviewer feels Dr. Mead describes rather too glowingly. This unusual culture contact occurred at a time when the conventional relations of colonial officials with their "wards" were wholly suspended. The Manus were getting a special kind of look at the West, and seeing much more than colonial administrators, missionaries, and anthropologists could (or perhaps would) ever have allowed them to see. With peace, the Manus began to reorganize their culture in new ways. Dr. Mead describes well the almost messianic cults which arose, symbolic validations of the people's desire to recapture and retain what they had seen and learned and possessed, once the troops (and their equipment) were gone, and the colonial officials and missionaries (and anthropologists) were back again. Out of the war's aftermath, and under the leadership of a gifted native—whose personal attributes seemed to this reviewer to have been given an importance out of all proportion to his historic rôle—the Manus have been remaking their society, complete with committees, democratic ferment, a "bank," and other things Western. The reign of the ghosts is gone forever, as are the overriding fear and the bitter economic competition.

Dr. Mead speaks out strongly for the new culture and reveals clearly her preference for it over the old. Yet she emphasizes that such a vast change-over has meant the frequent slipping and grinding of gears. Particularly with regard to religion, she stresses her feeling that the modern version of Christianity serves no better purpose for the Manus than did the rule of the ghosts. Institutional "vacuums" and new misunderstandings have arisen, since the whole panoply of Western society could not be fully presented to the Manus and many Western institutions which grew out of social needs in our own history have not been tidily transferred. Withal, the author plumps for the new; expresses the hope that in the hands of the young the new will not too soon become fixed and inflexible as the old was. Though Dr. Mead is reminded by this potentiality for rigidity of America's second generation, one senses she may have Soviet or Israeli youth at the back of her mind as well.

The photographs are superb, the typography poor; the style is oratorical and often gratuitously dramatic, but this is a book, like all those of its author, well worth reading.

SIDNEY W. MINTZ

ADDITIONAL BOOKS RECEIVED

The Patient Speaks. By Harold A. Abramson, M.D., Associate Attending Physician and Chief of the Allergy Clinic, Mount Sinai Hospital, New York; Research Psychiatrist, Biological Laboratory, Cold Spring Harbor; Consultant, Huntington Hospital; Assistant Clinical Professor of Physiology, Columbia University; Consultant (Psychology), Department of the Army. New York, Vantage Press, 1957. 273 pp. $3.50.

Sixth Report on Institutional and Special Purpose Research Grants of the American Cancer Society; September 1954-August 1955. Edited by the American Cancer Society, Inc. New York, 1956. 572 pp.

Diagnostic Procedures for Virus and Rickettsial Diseases. Edited by the American Public Health Association, New York. 1956. 2d ed. 596 pp. $7.50.

The Person Behind the Disease. By Julius Bauer, M.D., F.A.C.P., Clinical Professor of Medicine, College of Medical Evangelists, Los Angeles; Senior Attending Physician, Los Angeles County General Hospital; Consultant in Medicine, Long Beach Veterans Administration Hospital. New York, Grune & Stratton, Inc., 1956. 122 pp. $3.50.

Soybeans for Health, Longevity and Economy. By Philip S. Chen, Ph.D., Professor of Chemistry, Atlantic Union College. South Lancaster, Massachusetts, The Chemical Elements, 1956. 222 pp. $3.00.

Services for Children with Vision and Eye Problems. By Committee on Child Health of the American Public Health Association and the National Society for the Prevention of Blindness. New York, The American Public Health Association, Inc., 1956. 108 pp.

Services for Children with Hearing Impairment. By Committee on Child Health of the American Public Health Association. New York, The American Public Health Association, Inc., 1956. 120 pp.

The Dissection of the Cat. By Bruce M. Harrison, Ph.D., Sc.D., Emeritus Professor of Zoology, The University of Southern California, Los Angeles. 3d ed. St. Louis, The C. V. Mosby Company, 1956. 217 pp. $3.50.

The American Arbacia and Other Sea Urchins. By Ethel Browne Harvey. Princeton, New Jersey, Princeton University Press, 1956. 231 pp. $6.00.

Environmental Physiology of Animals. By Harold S. Hoar, University of British Columbia. New York, Scholar's Library. 76 pp. $2.25.

The Rabbit in Experimental Physiology. By Harold M. Kaplan, Southern Illinois University. New York, Scholar's Library. 69 pp. $2.50.

Lakeside Lecture Series. By the Lakeside Laboratories. Milwaukee, Wisconsin, Lakeside Laboratories Inc. 15 pp.

Women of Forty. By M. E. Landau, M.D., F.R.C.S. New York, Philosophical Library, 1956. 49 pp. $2.50.

The Happy Life of a Doctor. By Roger I. Lee, M.D. Boston, Little, Brown and Company, 1956. 278 pp. $4.00.

A Workbook of Anatomy. By C. P. Martin and S. M. Banfill, McGill University. New York, Scholar's Library, 1956. 142 pp. $3.50.

Dictionary of Poisons. By Ibert and Eleanor Mellan. New York, Philosophical Library, Inc., 1956. 150 pp. $4.75.

Effects of Natural Selection on Human Genotypes. By L. D. Dunn, Institute for the Study of Human Variation, Columbia University, New York; Howard Levene, Institute for the Study of Human Variation, Columbia University, New York; R. B. MacConnell, Heredity Clinic, David Lewis Northen Hospital, Liverpool, England. Kenneth T. Morse, Editor-in-Chief. Annals of the New York Academy of Sciences, vol. 65, art. 1, pp. 1-32. New York, 1956. $1.25.

Some Protozoan Diseases of Man and Animals: Anaplasmosis, Babesiosis, and Toxoplasmosis. Kenneth T. Morse, Editor-in-Chief. Annals of the New York Academy of Sciences, vol. 64, art. 2, pp. 25-277. New York, 1956. $3.50.

Calcium and Phosphorus Metabolism in Man and Animals with Special Reference to Pregnancy and Lactation. Kenneth T. Morse, Editor-in-Chief. Annals of the New York Academy of Sciences, vol. 64, art. 3, pp. 279-462. New York, 1956. $4.00.

Epidemic and Endemic Diarrheal Diseases of the Infant. Kenneth T. Morse, Editor-in-Chief. Annals of the New York Academy of Sciences, vol. 66, art. 1, pp. 3-230. New York, 1956. $3.50.

On the Cell Model for Solutions. By Stuart A. Rice, Department of Chemistry, Harvard University, Cambridge, Massachusetts. Kenneth T. Morse, Editor-in-Chief. Annals of the New York Academy of Sciences, vol. 65, art. 2, pp. 33-54. New York, 1956. $1.25.

Nerve Impulse. Transactions of the Fifth Conference, September 20, 21, and 22, 1954. Edited by David Nachmansohn, Professor of Biochemistry, Columbia University College of Physicians and Surgeons. New York, The Josiah Macy, Jr., Foundation, 1956. 256 pp. $4.50.

Group Processes. Transactions of the Second Conference, October 9, 10, 11, and 12, 1955. Edited by Bertram Schaffner, M.D., University Seminar on Communications, Columbia University, New York. New York, The Josiah Macy, Jr., Foundation, 1956. 255 pp. $3.50.

Procedure in Taxonomy. By Edward T. Schenk and John H. McMaters. Stanford, California, Stanford University Press, 1956. 3d ed. 119 pp. $3.50.

Plant Physiology. By Meirion Thomas, M.A., F.R.S., F.R.S.E., Professor of Botany, King's College, Newcastle-upon-Tyne in the University of Durham. New York, Philosophical Library, Inc., 1956. 4th ed. 692 pp. $12.00.

Handbook of Histology. By Karl A. Stiles, Ph.D., Professor and Head of the Department of Zoology, Michigan State University. New York, The Blakiston Division, McGraw-Hill Book Company, 1956. 240 pp.

Atti del Terzo Congresso Internazionale, Venezia 1955—Vitamina A. Verona, Italy, Edizioni Valdegna, 1956. 631 pp.

Dictionary of Anthropology. By Charles Winick. New York, Philosophical Library, 1956. 579 pp. $10.00.

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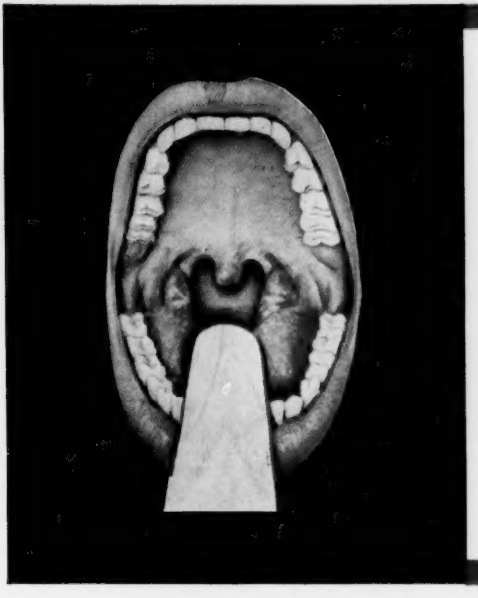

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1. Carter, C. H., and Maley, M. C.: Antibiotics Annual 1956-1957, New York, Medical Encyclopedia, Inc., 1957, p. 51.

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*Miller, J.B., et al.: Ann. Allergy, 12:611, Sept.-Oct., 1954.

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